MICROBIAL BASIS FOR THE DIFFERENTIAL ENHANCED DEGRADATION OF CIS- AND TRANS-1,3-DICHLOROPROPENE (1,3-D) IN SOILS WITH A HISTORY OF REPEATED FIELD APPLICATIONS

By

KEUN-YOOK CHUNG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1998

To my parents and my wife

ACKNOWLEDGMENTS

I would like to express my sincere thanks to my committee chairman, Dr. Li-Tse Ou. His philosophical guidance and expert knowledge in the area of biodegradation and microbial ecology have made this dissertation work possible. Sincere thanks are also extended to the other committee members, Drs. B.L. McNeal, P. Nkedi-Kizza, A.C. Wilkie, and W.B. Wheeler for their help and guidance. I also thank Drs. D.M. Sylvia and A.V. Ogram for their encouragement and care. Special thanks go to Dr. D.W. Dickson who provided a field site for my dissertation research.

I also want to express my endless appreciation to John Thomas for his continued and kind help throughout the entire period of my graduate studies, and especially for his expertise in the operation of complex analytical instruments such as HPLC, GC, Headspace-GC, and various computer systems. I will miss him wherever I go. My thanks are also extended to Dr. S.L. Trabue for his friendship and encouragement.

I would like to thank my parents and my parents-in-law in Korea for their encouragement and financial support, and

especially my wife, Hyung-Min Yoon, for her patience, encouragement and love. I feel sorry for not sitting and playing more with my son, Daniel (Min-Goo), and with my daughter, Michaela (Min-Jae). I also appreciate my brothers, my sister, and my brother- and sisters-in-law in my country for their encouragement. Especially I thank my elder brother, Keun-Chang Chung, in Korea very much for his continued encouragement.

Finally, I thank my undergraduate professors, Drs. Jae-Joung Kim, Chang-Soo Yuk, Jae-Goo Lee, and new faculty members Drs. Soon-Dal Hong and Young-Ki Kim in the Department of Agricultural Chemistry at Chung Buk National University of Korea, for encouraging me to pursue graduate studies in soil microbiology in the U.S.A.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

MICROBIAL BASIS FOR THE DIFFERENTIAL ENHANCED DEGRADATION OF CIS- AND TRANS-1,3-DICHLOROPROPENE (1,3-D) IN SOILS WITH A HISTORY OF REPEATED FIELD APPLICATIONS

By

Keun-Yook Chung

May, 1998

Chairman: Dr. Li-Tse Ou
Major Department: Soil and Water Science

The fumigant 1,3-dichloropropene is considered to be a potential alternative to methyl bromide. The degradation rates of cis- and trans-1,3-D in surface (0-15 cm), shallow subsurface (15-30 cm), and deep subsurface (30-45 cm) soils collected from treated and untreated sites at the Green Acres Farm near Gainesville, FL, were determined after three successive annual applications of 1,3-D to the treated site from 1995 to 1997. This study showed that degradation rates of cis- and trans-1,3-D progressively increased with an increase in the number of annual applications, with enhancement of trans-1,3-D degradation being greater than for cis-1,3-D. In nonenhanced soils, the degradation rates of the two isomers were similar.

The enhancement was greater for the surface (0-15 cm) and shallow subsurface (15-30 cm) soils than for the deep subsurface (30-45 cm) soil. The enhancement lasted ≈2 years after annual field application of 1,3-D had ceased. A single field reapplication of 1,3-D to a treated site that had not been treated for 2 years resulted in resumed differential enhanced degradation of cis- and trans-1,3-D.

A mixed bacterial culture capable of degrading 1,3-D was isolated from an enhanced soil sample collected from the treated site. Similar to the enhanced soil, the mixed culture degraded trans-1,3-D faster than cis-1,3-D. The mixed culture degraded cis- and trans-1,3-D only in the presence of a suitable second substrate. Thus, this mixed culture could not utilize cis- and trans-1,3-D as a sole source of carbon for growth, and degradation of cis- and trans-1,3-D was a cometabolic process. Second substrates that had the capacity to stimulate the degradation included soil leachate, tryptone, and tryptophan. Other substrates tested, including soil extract, glucose, and yeast extract, failed to stimulate the degradation of the two isomers. The mixed culture was composed of at least four species of bacteria.

In conclusion, differential enhanced degradation of cis- and trans-1,3-D in soils progressively increased during the three consecutive annual applications of 1,3-D, and microorganisms were involved in the degradation process.

INTRODUCTION

Overview of Pesticide Degradation in Soil

Types and Usage of Pesticides

Pesticides are at present indispensable tools for the protection of economically important crops from damage by weeds, insects, fungi, and nematodes in modern agriculture. Approximately 600 different pesticides were registered for use in the U.S.A. in 1989 and millions of kilograms are applied to crops each year, occupying a world market estimated at greater than 20 billion dollars annually (McDougall and Woodburn, 1989).

Most pesticides are synthetic organic chemicals, which are classified and characterized in terms of the types of pests they target (Hassall, 1982). The four major pesticide groups include herbicides, insecticides, fungicides, and nematicides, designed for the control of weeds, insects, plant pathogenic fungi, and nematodes, respectively.

Transport of Pesticides in Soils

Pesticides should be persistent for an appropriate time to effectively control target pests in soils. However, pesticides in soils are subject to transport (volatilization and leaching) as well as transformation (degradation)(Jury and Ghodrati, 1989). Volatilization, transporting pesticides

from soil to the atmosphere, is affected by vapor pressure of the chemical and temperature of the soil. Leaching, the transport from soil to groundwater, is governed by soil-specific sorption and desorption coefficients of the chemicals. At present, octanol/water coefficients (Kow values) and organic carbon partition coefficients (Koc values) are critical parameters typically related to sorption and desorption processes.

Degradation Rates and Environmental Factors

Degradation rates of pesticides in soils provide another important type of information for predicting probable fates in the environment. Ou et al. (1982) considered a number of physical, chemical, and biological factors that might influence the degradation rates of pesticides in soils. They included soil type, soil temperature, soil pH, soil moisture content, organic matter content, and microbial populations, with soil temperature and moisture content considered to be among the most important environmental factors influencing pesticide degradation in soils (Smith and Walker, 1977; Walker, 1974, 1976a, 1976b, 1978; Walker and Smith, 1979).

Degradation and Metabolism of Pesticides

Abiotic and biotic transformations are the most important mechanisms for detoxifying pesticides in soils. The transport mechanism translocates a pesticide from one soil compartment to another without appreciable decrease in

the total mass of chemical. Abiotic transformations are sometimes involved in the partial degradation of pesticides, resulting in accumulation of metabolites in soils (Alexander, 1994). Biological degradation, however, may lead to complete detoxification; i.e., complete mineralization to CO_2, H_2O, and inorganic ions (Alexander, 1981). In this respect, it should be noted that soil is a biologically diverse and dynamic ecosystem. Soil microorganisms such as bacteria and fungi continually perform a key role in the recycling of major nutrients from the organic fraction of the soil (Alexander, 1977). Bacteria in particular possess a physiologically versatile metabolic function on a variety of natural and synthetic organic compounds, including pesticides in soils (Palleroni, 1995).

The microbial metabolism of a pesticide can be categorized into two distinctive processes, mineralization and cometabolism. Mineralization of a pesticide to inorganic compounds such as CO_2, H_2O, and inorganic ions in soils is a normal consequence of microbial activity (Alexander, 1981). This process can support microbial growth, using the pesticides as carbon and energy sources. Cometabolism, on the other hand, does not directly provide carbon and energy for growth during the degradation process (Horvath, 1972).

Many natural and synthetic organic chemicals are degraded through various metabolic reactions (Alexander, 1977, 1994), including:

1. Hydrolysis. This is the major critical mechanism which is generally involved in the degradation of pesticides. Examples include the transformation of carbofuran to carbofuran phenol (Ou et al., 1982; Trabue et al., 1997; Turco and Konopka, 1990), parathion to p-nitrophenol (Sethunathan et al., 1977), carbaryl to 1-naphthol (Chapalamadugu and Chaudhry, 1991), and fenamiphos to fenamiphos phenol (Ou, 1991; Ou et al., 1994).

2. Oxidation of the sulfur in a molecule. Those chemicals containing a sulfur molecule generally can be oxidized to sulfoxide and further to sulfone. For example, aldicarb can be oxidized to aldicarb sulfoxide and further to aldicarb sulfone (Ou et al., 1985a, 1985b), and fenamiphos to fenamiphos sulfoxide and eventually to fenamiphos sulfone (Ou, 1991; Ou et al., 1994).

3. Addition of an oxygen to a double bond, with the resulting epoxides frequently being recalcitrant to microbial attack and consequently persistent. Examples include the oxidation of heptachlor to heptachlor epoxide (Duffy and Wong, 1967; Elsner et al., 1972), and of aldrin to dieldrin (Lichtenstein and Schultz, 1960).

4. Oxidation of an amino group (NH_2) to a nitro group: RNH_2-> RNO_2.

5. Addition of a hydroxyl group to a benzene ring. This is an oxidation reaction, involving a dioxygenase or monooxygenase that catalyzes the reaction. For example,

transformation of 2,4-D (2,4-dichlorophenoxy acetic acid) to 2,4-dichlorophenol is catalyzed through α-ketoglutarate dependent 2,4-D dioxygenase (Fukimori and Hausinger, 1993a, 1993b; Suwa et al., 1996; Top et al., 1996).

6. Dehalogenation. Many pesticides and some industrial chemicals are halogenated compounds. Generally, the halogens may be replaced by H (reductive dehalogenation). For example, DDT (2,2-p-chlorophenyl)-1,1,1-trichloroethane) can be transformed to DDD (2,2-p-chlorophenyl)-1,1,1-dichloroethane) (Guenzi and Beard, 1967, 1968, 1976). They can also be replaced by OH (hydrolytic dehalogenation). An example is the transformation of 1,3-D (1,3-dichloropropene) to 3-CAA (3-chloroallyl alcohol) (Roberts and Stoydin, 1976). This reaction mechanism will be discussed in a subsequent section on the degradation of halogentated hydrocarbon compounds.

7. Reduction of a nitro group (NO_2) to an amino group (NH_2). An example is the transformation of parathion to amino parathion in soil (Barik et al., 1979; Lichtenstein and Schultz, 1964).

8. Replacement of a sulfur with an oxygen. Many organophosphate insecticides contain P=S bonds in their structure, and this portion is typically modified during the initial reaction to P=O, which tends to make the metabolite more toxic. An example is the transformation of parathion to

paraoxon, which is more toxic than parathion (Munnecke and Hsieh, 1976; Sethunathan and Yoshida, 1973).

9. Metabolism of side chains. Hydrocarbon chains connected to the benzene rings of pesticides are often removed before the ring is broken down, with β-oxidation generally involved in this metabolism of side chains. Eventually, the side chains are metabolized to CO_2. An example is the transformation of 2,4-DB (2,4-dichlorophenoxy butyric acid) and MCPB (4-(2-methyl-4-chlorophenoxy)butyric acid)(Thomson, 1986).

10. Ring cleavage. The first step of ring cleavage involves hydroxylation of the ring by a monooxygenase or dioxygenase to form a catechol compound, which is then subject to ring cleavage via ortho or meta fission involving a dioxygenase, and eventually is converted to common biochemical intermediates, such as succinate and acetate (Voet and Voet, 1990). Thus, relatively toxic and persistent halogenated pesticides including aromatic benzene rings in their structures should eventually be completely broken down into inorganic molecules such as CO_2, H_2O, and halogen elements (Cl, Br, F, etc.)(Bayly and Barbour, 1984).

Biological mechanisms are extensively involved in the above reactions, and chemical mechanisms can be partially involved in some reactions as well. However, biological reactions usually catalyze the degradation of pesticides in soils at a more rapid rate than by chemical reactions

through the enzymes (either extracellular or intracellular) produced by microorganisms (Alexander, 1994).

Enhanced Degradation of Pesticides

Historical Aspects of Enhanced Degradation of Pesticides in Soils

In recent years, enhanced (accelerated) degradation of pesticides in soils has attracted considerable interest and attention among microbiologists and agricultural scientists. This phenomenon was first observed nearly 50 years ago by Audus (1951) for the phenoxyalkanoic acid herbicides, 2,4-D and MCPA (2-methyl-4-chlorophenoxy acetic acid), in soil suspensions using a soil perfusion apparatus in the laboratory.

Nearly thirty years later, the thiocarbamate herbicide EPTC (S-ethyl dipropyl thiocarbamate) and the carbamate insecticide carbofuran were the first to be positively linked to crop failure due to enhanced degradation of the chemicals in a New Zealand soil and an Illinois soil, respectively (Felsot et al., 1981; Rahman et al., 1979). These researchers found that repeated field applications of the chemicals had resulted in enhanced degradation in soils, causing poor pest control and corresponding crop yields. Consequently, their studies triggered a great interest in enhanced degradation of pesticides.

In a short period of time following the above two publications, EPTC was found to be ineffective in controlling target weeds due to enhanced degradation in a

Nebraska soil (Obrigawitch et al., 1980), a Wisconsin soil (Schuman and Harvey, 1980), and an Iowa soil (Gunsolus and Fawcett, 1980). All the soils had previous histories of exposure to the chemical.

Carbofuran also was reported to offer inadequate protection against certain soil insects in an Iowa soil (Read, 1983), a Kansas soil (Wilde and Mize, 1984), and an eastern Canada soil (Tollefson, 1986) with histories of carbofuran use. In addition to these compounds, other pesticides including: carbamate insecticides, carbaryl (Rajagopal et al., 1986) and aldicarb (Read, 1987; Suett and Jukes, 1988); organophosphorous pesticides, isofenfos (Racke and Coats, 1987) and fenamiphos (Ou, 1991; Ou et al., 1994); and fungicides including iprodione (Martin et al., 1990; Walker et al., 1986) and vinclozolin (Cain and Mitchell, 1996; Walker et al., 1986), were reported to exhibit similar phenomena in several countries.

Disadvantages and Advantages of Enhanced Degradation

Disadvantages of enhanced degradation. Enhanced degradation of pesticides often results in poor control of target pests. Consequently, crop production can be greatly reduced. Enhanced degradation related to pest-control problems has been most thoroughly studied with the methyl-carbamate insecticides, carbofuran, carbaryl, and aldicarb, and with the thiocarbamate herbicides EPTC, butylate, and vernolate (Felsot, 1989).

A rapid decrease in carbofuran concentration was found to be associated with poor control of the insect phylloxera in a vineyard planted in gravelly loam soil in Canada, of pH 6.0 (Willams et al., 1976). Subsequently, Felsot et al. (1982) demonstrated a relationship between the enhanced degradation of carbofuran and its efficacy to control rootworms. They showed that a low population density of corn rootworm was found to be correlated with a high percentage of carbofuran recovery. The persistence of carbofuran was the shortest in those soils characterized by both carbofuran-use histories and rootworm control problems. Also, Niemczyk and Chapman (1987) reported that enhanced degradation of the organophosphorous insecticide isofenfos occurred in turfgrass thatch and underlying soil where poor control of larvae was observed. Recently, Ou et al. (1994) reported that enhanced degradation of the nematicide fenamiphos may be related to poor nematode control of golf-course turfgrass.

Advantages of enhanced degradation. Even though enhanced degradation may result in reduced pesticidal efficacy and, consequently, poor crop yield, this is offset by reduced chance for pesticide contamination of groundwater. Enhanced degradation of triazine herbicides such as atrazine does not occur in soils, so their residues are frequently detected in groundwater. Carbamates and organophorous compounds are rarely detected in groundwaters,

however. Furthermore, the principles of enhanced degradation can be applied to remediate contaminated soils and groundwaters.

Enzymes are responsible for the degradation of pesticides in soils (Alexander, 1994). It is difficult to directly isolate active enzymes intact from soil. So, at present, their role in pesticide degradation is not clear. Most of these enzymatic transformations depend on the presence of viable organisms but some enzymes remain active extracellularly for long periods of time (Skujins, 1976). There is some evidence that extracellular soil enzymes catalyze the hydrolysis of organophosphorous insecticides at a rapid rate (Getzin and Rosefield, 1968; Gibson and Burns, 1977; Iwata et al., 1977).

Generally, information on bioremediation of pesticides has been limited to the remediation of soils contaminated with high concentrations of the organophosphorous pesticides, particularly, parathion and diazinon (Barles et al., 1979; Daughton and Hsieh, 1977). In both studies, decontamination of parathion from a parathion spillage site and a disposal site was seeded with a highly adapted parathion-degrading bacterium. Barles et al. (1979) reported that the adapted microorganism had the capacity to degrade up to 25% of the applied amount of parathion within 35 days. Furthermore, Barik and Munnecke (1982) further demonstrated that direct application of a hydrolytic enzyme to a field

soil resulted in degradation of concentrated diazinon. Instead of using live adapted microorganisms and free enzymes, Munnecke (1979) employed an immobilized parathion-hydrolase and found that the immobilized enzyme detoxified parathion and eight other organophosphate pesticides in waste water more effectively than microorganisms and free enzymes. Caldwell and Raushel (1991) demonstrated that a nylon-based immobilized phosphotriesterase from *Pseudomonas diminuta* was effective in detoxifying the organophosphate pesticide, paraoxon, in the laboratory.

However, in spite of such promising potential applications, very few microorganisms and enzymes have been tested as possible tools for the detoxification of pesticides. Research in this area can contribute new methods for pollution control (Nannipieri and Bollag, 1991). Therefore, further extensive field applications of adapted microorganisms or free or immobilized enzymes which can degrade a number of toxic pesticides are needed to verify the feasibility of decontaminating soils under in-situ conditions.

Mechanisms of Enhanced Degradation

Biological basis. It is well known that some pesticides are degraded more rapidly by microbial populations adapted following repeated application of the pesticides to the same soil, resulting in reduction of adaptation periods (Alexander, 1994; Racke and Coats, 1990). This process is

termed enhanced degradation, or accelerated degradation. The assumption that enhanced degradation is largely a biological phenomenon has been experimentally verified through soil sterilization, including the use of microbial inhibitors (Rahman and James, 1983), gamma irradiation (Kaufman and Edwards, 1983), and autoclaving (Rahman and James, 1983). Further evidence is derived from increased $^{14}CO_2$ evolution in enhanced soil treated with ^{14}C-labelled pesticides (Obrigawitch et al., 1982; Ou, 1991; Wilson, 1984).

Several mechanisms have been proposed to explain the adaptation of microorganisms involved in such enhanced degradation. The adaptation period may signify the time for an initially small microbial population to reach sufficient population density, resulting in a rapid decrease in concentration of the pesticide (Ventullo and Larson, 1986; Wiggins et al., 1987). Alternatively, the adaptation period may be the time required for microorganisms to produce sufficient enzymes to initiate degradation of the applied pesticide (Stephenson et al., 1984). It could also reflect the time for mutation or the exchange of genetic materials among the soil microbial populations to yield a phenotypic microorganism which can use the pesticide (Schmidt et al., 1983; Van der Meer et al., 1992; Varma et al., 1976) and/or the acquisition of extraordinary degradative capability through transconjugation and plasmid transfer (Harder, 1981). Plasmids, transposons, and insertion-sequence (IS)

elements are implicated in providing the mechanisms for adaptation of microorganisms to rapid pesticide degradation (Clarke, 1984). Alternatively, the time for the destruction or removal of microbial inhibitors, or for the utilization of more readily metabolizable pesticides, may also result in adaptation (Atlas and Bartha, 1972; Kim and Mayer, 1986).

In summary, microbial adaptation can occur through one or more of the following three mechanisms: 1) induction or derepression of the degradative enzymes; 2) selection of new metabolic capabilities produced by genetic changes; and/or 3) an increase in the number of microorganisms able to transform the chemicals (Spain et al., 1980). The third mechanism may be actually a combination of the first two mechanisms.

Degradation patterns in enhanced and nonenhanced soils. Repeated applications of a pesticide to a field soil may result in more rapid degradation (Camper et al., 1987; Felsot et al., 1981; Kaufman et al., 1985) and different degradation pathways (Dick et al., 1990; Ou et al., 1994; Trabue et al., 1997) compared to that of untreated soil. It is known that the degradation pathways of pesticides in enhanced soil are more efficient and simpler than those in nonenhanced soil (Chung and Ou, 1996; Ou et al., 1994; Trabue et al., 1997; Turco and Konopka, 1990). For example, fenamiphos (FS) in enhanced soils is rapidly oxidized to fenamiphos sulfoxide (FSO), which is then rapidly hydrolyzed

in turn to fenamiphos sulfoxide phenol (FSO-OH) (Fig. 1). The resulting phenolic compound (FSO-OH) is rapidly mineralized to CO_2, H_2O, and SO_4. By contrast, fenamiphos in nonenhanced soils undergoes hydrolysis to fenamiphos phenol (FS-OH) and slow oxidation to FSO which is then slowly oxidized to fenamiphos sulfone (FSO_2). Two major oxidation products, FSO and FSO_2, are slowly hydrolyzed (respectively) to FSO-OH and FSO_2-OH, and then eventually to CO_2, H_2O, and SO_4 (Chung and Ou, 1996; Ou et al., 1994).

A second example is carbofuran. Carbofuran in enhanced soils is rapidly hydrolyzed to carbofuran phenol and methyl amine, which are in turn rapidly mineralized to CO_2, NO_3, and H_2O (Trabue et al., 1997)(Fig. 2). Carbofuran in nonenhanced soils undergoes both oxidation and hydrolysis simultaneously at slow rates, to hydroxycarbofuran and carbofuran phenol, respectively (Fig. 2). 3-Hydroxy-carbofuran is then oxidized to 3-ketocarbofuran, and 3-hydroxycarbofuran and 3-ketocarbofuran are then further hydrolyzed to 3-hydroxycarbofuran phenol and 3-keto-carbofuran phenol, respectively. The three phenolic hydrolysis products are eventually slowly mineralized to CO_2, NO_3, and H_2O (Ou et al., 1982). This fact is supported by the findings of Turco and Konopka (1990). They also reported that the degradation of carbofuran in enhanced soil

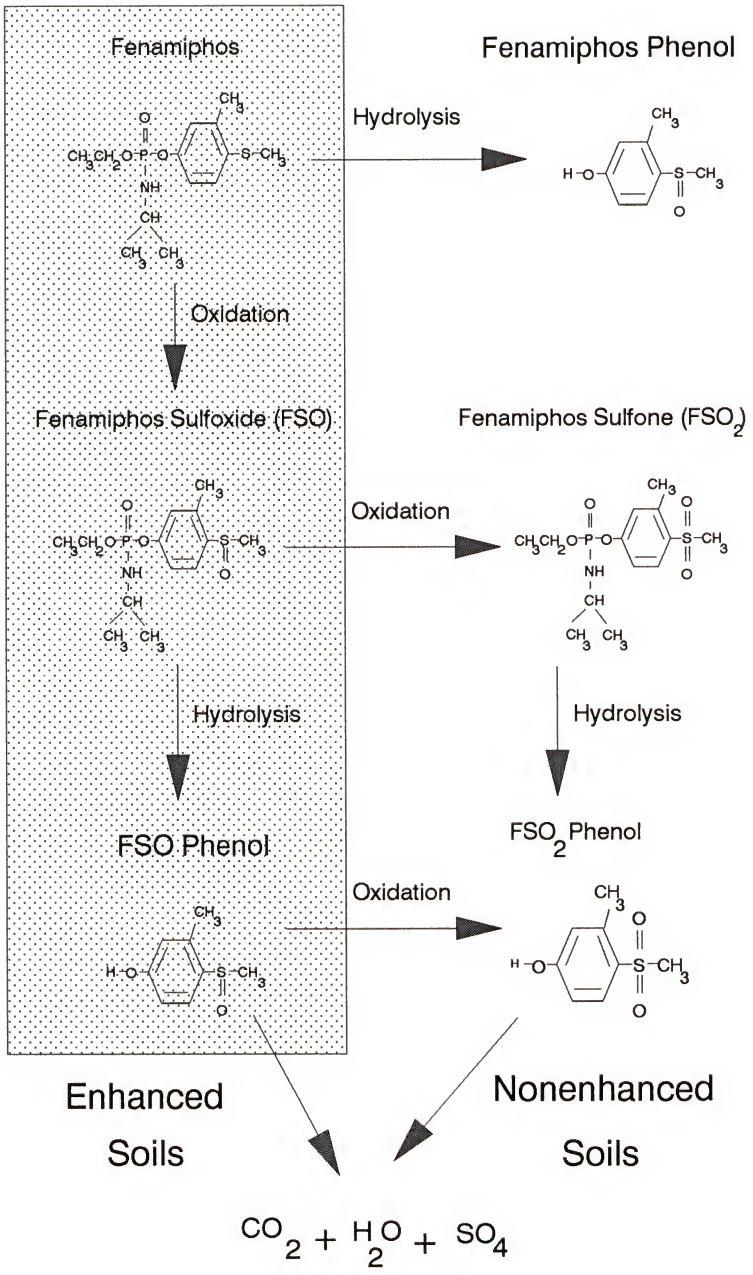


Figure 1. Proposed degradation pathways of fenamiphos in enhanced and nonenhanced soils (Chung and Ou, 1996; Ou et al., 1994).

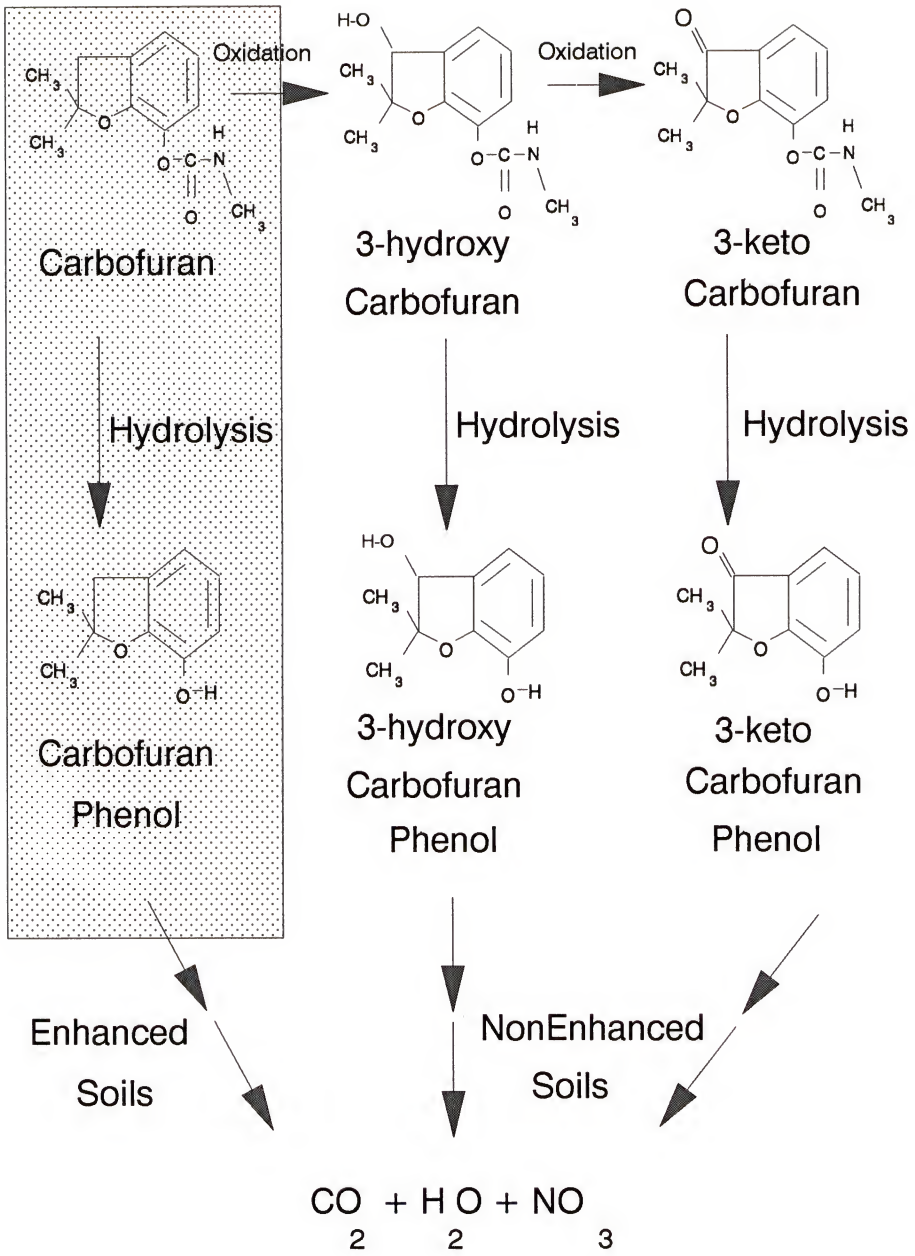


Figure 2. Proposed degradation pathways of carbofuran in enhanced and nonenhanced soils (Ou et al., 1982; Trabue et al., 1997).

proceeded mainly through biological hydrolysis to carbofuran phenol, which in turn was then rapidly degraded to its final oxidation products, CO_2, NO_3, and H_2O.

EPTC provides another example of differing degradation pathways in enhanced and nonenhanced soils (Fig. 3). In enhanced soils, EPTC undergoes hydroxylation to form N-depropyl EPTC, which is the major metabolite. However, EPTC in nonenhanced soil undergoes both sulfoxidation and hydroxylation, resulting in a large production of EPTC sulfoxide (Dick et al., 1990).

Induction and duration of enhanced degradation. The number of applications to field soils through which enhanced degradation of pesticides occurs depends on the nature of the pesticides. A single field application of some pesticides may result in enhanced degradation of the chemicals. These pesticides include carbofuran (Eagle, 1986; Getzin and Shanks, 1990; Harris et al., 1988; Trabue et al., 1997), fenamiphos (Ou, 1991), EPTC (Obrigawitch et al., 1982; Rahman et al., 1979; Schuman and Harvey, 1980), and butylate (Schuman and Harvey, 1980). On the other hand, enhanced degradation has not been observed for some pesticides, despite repeated field applications of the chemicals. For example, enhanced degradation of DDT has never been observed, despite extensive use in many countries prior to 1970 and its continued use in several tropical countries. Generally, however, an increase in number of

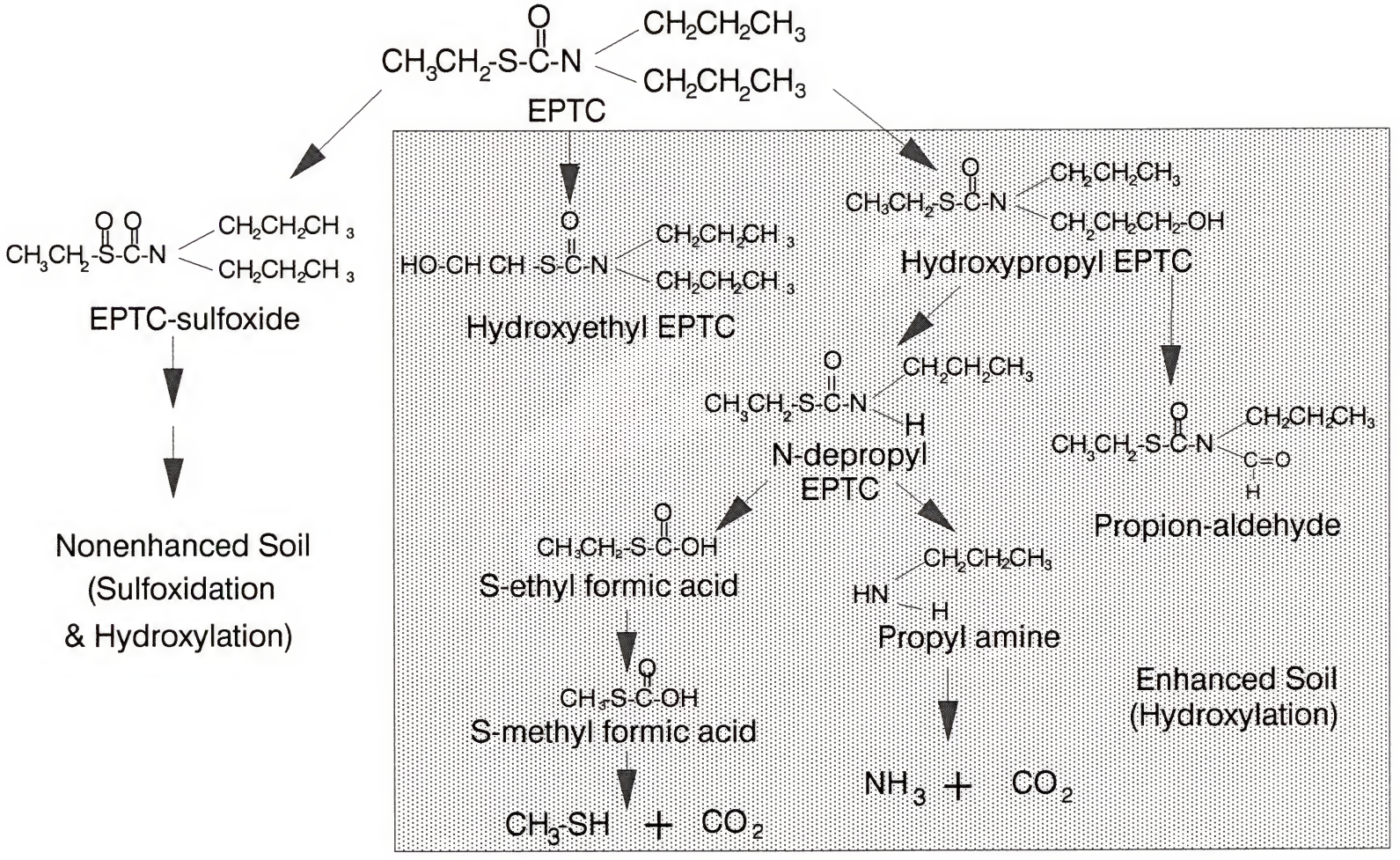


Figure 3. Proposed degradation pathways of EPTC in enhanced and nonenhanced soils (Dick et al., 1990).

field applications of a pesticide results in a progressive increase in the degradation rate of the pesticide in the soil (Avidov et al., 1988; Camper et al., 1987; Ou et al., 1994; Racke and Coats, 1990; Trabue et al., 1997).

The degree of enhancement after repeated field applications of a pesticide may depend on soil type, climate conditions, and type(s) of pesticide-degrading microorganism evolved in response to the pesticide. Obrigawitch et al. (1983) observed that butylate degradation was enhanced increasingly with each successive application during a period of three annual field applications of the chemical to a Kennebec silt loam soil in Nebraska. However, Dowler et al. (1984) found the degradation rate of butylate in enhanced soil (Tifton loamy sand) of Georgia did not change whether there was only one field application or five consecutive annual applications of butylate. Usually, one or two prior field treatments of a pesticide suffice to initiate enhanced degradation of the chemical(Bailey and Coffey, 1985; Roeth, 1984).

The duration of enhanced degradation for a pesticide varies from soil to soil and may be influenced by physical, chemical, and biological characteristics of the soil (Bean et al., 1988). Therefore, long-term field studies are needed for accurate determination of the durations of enhancement of a pesticide under various soil conditions after field applications of the pesticide have ceased. At present,

little information on this subject is available. Furthermore, climate could be an important factor influencing the duration of enhancement. Enhancement for a single field application of fenamiphos to a northeast Florida soil (temperate zone) lasted more than 3 years but less than 4 years (Ou, 1991). In contrast, duration of the enhanced degradation period for fenamiphos in banana plantation soils located in tropical regions lasted only 18 months (J.P.E. Anderson, personal communication). Enhanced degradation of MCPA in a temperate-zone soil in England lasted 5 years after the last field applications of the herbicide (Fryer et al., 1980). Although these studies involved a variety of soils, they do suggest that climate significantly affects enhancement duration.

Cross adaptation reactions. Cross-enhancement or cross adaptation is a term used to explain the enhanced degradation of one pesticide after pretreatment of the soil with another. Carbofuran, a carbamate insecticide, and fensulfothion, an organophosphate, have been known to have cross-adaption between the two classes of pesticides (Read, 1983). Usually the two pesticides involved are structurally related analogs (Felsot, 1989; Roeth, 1986), with many structural analogs existing among the carbamate and organophosphate pesticides. Cross-enhancement among the carbamate pesticides is particularly common (Harris et al., 1984; Racke and Coats, 1988; Smelt et al., 1987; Suett,

1987). On the other hand, enhanced degradation of organophosphorous pesticides is more specific, even though just one study suggested cross-adaptation between chlorpyrifos and terbufos (Horng and Kaufman, 1987). Obrigawitch et al. (1983) reported that degradation of the thiocarbamate herbicides vernolate and butylate was enhanced in soils pretreated with EPTC, which is also a thiocarbamate. Wilson (1984) reported that the degradation of EPTC was enhanced in soils previously treated for 3 years with EPTC, vernolate, and butylate. Vernolate also was degraded more rapidly in soils pretreated with EPTC or vernolate, but the degradation of butylate was much faster in soils pretreated with butylate than in soils pretreated with EPTC or vernolate. However, the degradation of the thiocarbamate cycloate was not enhanced in soils pretreated with EPTC, vernolate, or butylate.

Strategies for Preventing Loss of Pesticidal Efficacy from Enhanced Degradation

Two general strategies for overcoming the enhanced degradation of pesticide have been proposed and verified via actual agronomic practices (Felsot, 1989). They are thus both operational and technological strategies.

Operational strategies, which rely on management techniques to counteract the enhanced degradation, include conservation of pesticides (Turpin and York, 1981), crop rotation (Suett, 1986), proper calibration of equipment (Ellis, 1982), altered timing of applications (Steffey et

al., 1987), and chemical rotation (Read, 1986; Rudyanski et al., 1987; Zublena et al., 1987). On the other hand, technological strategies require changes in pesticide formulation or structure, use of extenders and inhibitors, and new formulation technology (Felsot, 1989). Use of extenders and inhibitors, which may be included in the pesticide formulations, increases persistence of the pesticides by preventing or slowing down biodegradation of the pesticides during certain periods of time.

It has been reported that fonofos, a soil-applied organophosphate insecticide, can increase EPTC persistence and efficacy in soils with previous EPTC exposure (Camacho et al., 1981; Harvey and Schuman, 1981). Also, it has been demonstrated that dietholate, an organophosphate compound, can increase soil persistence and efficacy of EPTC and butylate (Camacho et al., 1981; Gunsolus and Fawcett, 1981; Harvey and Schuman, 1981; Obrigawitch et al., 1982; Rahman and James, 1983). However, repeated applications of EPTC with either dietholate or fonofos eventually caused the enhanced degradation of EPTC, resulting in reduction of its efficacy (Gunsolus and Fawcett, 1981; Roeth, 1984). Felsot (1989) considered that operational strategies, rather than technological approaches, were the most desirable for long-term management of the enhanced degradation problems of pesticides.

Degradation of Halogenated Hydrocarbon Compounds

Halogenated hydrocarbons are an important class of chemicals used for many industrial applications (Chaudhry and Chapalamadugu, 1991). They are used as pesticides, plastics, solvents, and degreasers. Despite their industrially beneficial uses, some have caused serious environmental damage including contamination of air, soil, and groundwater, killing of or injuries to nontarget organisms, depletion of the stratospheric ozone layer, and long-range transport. Many are suspected carcinogens to humans, and persistent in soils and waters (Fetzner and Lingens, 1994). Consequently, the public is concerned about possible adverse effects on the quality of life due to these compounds.

Most halogenated compounds fall into two main groups. The first group of these chemicals includes short-chain halogenated aliphatic hydrocarbons such as trichloroethylene (TCE), ethylene dibromide (EDB), methyl bromide (MeBr), chloroform, and 1,3-dichloropropene (1,3-D). The second group includes halogenated aromatic compounds such as DDT, polychlorinated biphenyls (PCBs), chlorophenols, chlorobenzenes, chlorobenzoates, chlorotoluenes, and chlorophenoxyacetates.

Since 1,3-D is a short-chain halogenated aliphatic hydrocarbon, subsequent discussion will be principally

focused on the short-chain halogenated aliphatic hydrocarbons.

Mechanisms of Dehalogenation

The carbon-halogen bond is strong or recalcitrant to breakdown, due to the increased electronegativity of the substituents, and halogenated compounds with one or at most a few substituents are thought to be more readily degradable than the corresponding polyhalogenated compounds (Reineke, 1984). Of the possible carbon-halogen bonds, the C-F bond is the strongest and results in more recalcitrance, comparable to a carbon-hydrogen bond, and high dissociation energy. On the other hand, the C-I bond is the weakest carbon-halogen bond and is much less recalcitrant. Carbon-halogen bonds can be broken either by biological (enzymatic) or chemical dehalogenation to form unstable intermediates, with biological mechanisms generally being faster than chemical mechanisms under the same condition. Many dehalogenating enzymes are involved in catalyzing the removal of halogen elements.

Fetzner and Lingens (1994) suggested that seven mechanisms can be involved in biological dehalogenation:

1. Reductive dehalogenation. Reductive dehalogenase can catalyze replacement of the halogen substituent on aliphatic or aromatic ring carbons by hydrogen atoms.

2. Oxygenolytic dehalogenation. Monooxygenase or dioxygenase can be involved in incorporating one or two

atoms of molecular oxygen into the substrate, concurrently removing halogen atoms.

3. Hydrolytic dehalogenation. Hydrolytic dehalogenase can catalyze the replacement of halogen atoms by the hydroxyl (OH) groups of water.

4. Thiolytic dehalogenation. A dehalogenating glutathione S-transferase, which is produced by dichloromethane-utilizing bacteria, can catalyze the formation of a S-chloromethyl glutathione conjugate, with concomitant release of chlorine atoms.

5. Intramolecular substitution. Intramolecular nucleophilic displacement accelerates the formation of epoxides, which are involved in the dehalogenation of vicinal haloalcohols.

6. Dehydrohalogenation. The concurrent elimination of HCl from halogen compounds can result in the formation of a double bond.

7. Hydration. Hydratase enzyme can catalyze the step of adding a water molecule to form unstable intermediates during the breakdown of halogenated compounds.

Because of the widespread use of halogenated hydrocarbons in many industries and their persistence in conjunction with inadequate past techniques for handling, storage, and disposal of these chemicals, halogenated hydrocarbons have become ubiquitous environmental pollutants. In fact, these compounds are the most frequent

groundwater contaminants in the United States (Baker and Herson, 1994). These chemicals are toxic to humans, and many of them are suspected carcinogens. Consequently, the public is concerned over the contamination of these chemicals in drinking water (Geiger and Molner-Kubica, 1977; Petura, 1981).

In summary, trichloroethylene (TCE) and methyl bromide (MeBr) (Fig. 4) each have structural similarity (i.e., short-chain halogenated aliphatic hydrocarbons) and are each degraded by cometabolism. 1,3-Dichloropropene (1,3-D) is another short-chain halogenated aliphatic hydrocarbon. Therefore, an interesting question is whether the biological degradation patterns of 1,3-D (Fig. 4) are the same as those of TCE and MeBr. The degradation of 1,3-D is discussed in the section following those for TCE and MeBr.

Trichloroethylene

Trichloroethylene belongs to a family of synthetic chlorinated aliphatic hydrocarbons manufactured as industrial solvents having a greatly reduced potential for fire or explosion (Ensley, 1991). It is widely used for degreasing and cleaning metals and electronic components. Because of frequent detection in drinking water aquifers (Love and Eilers, 1982), TCE has been classified as a priority pollutant in the U.S.A. In addition, TCE is the most frequently reported contaminant at hazardous waste

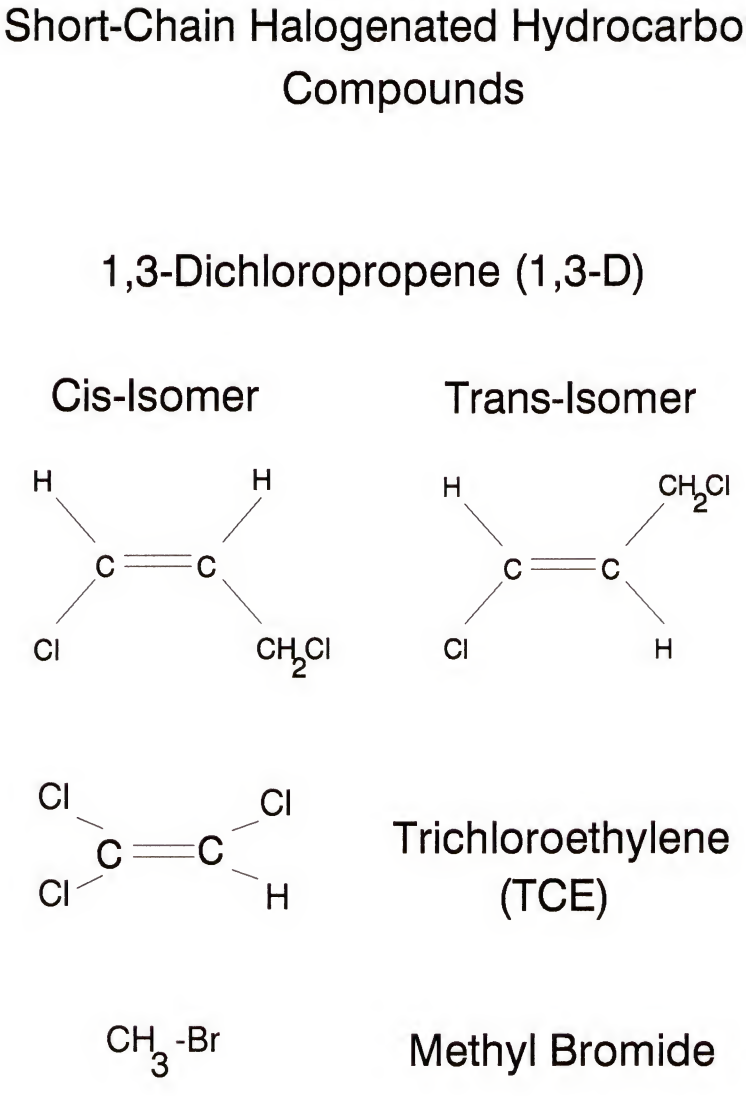


Figure 4. Chemical structures of short-chain halogenated aliphatic hydrocarbon compounds.

sites on the National Priority List of the U.S. Environmental Protection Agency (USEPA, 1985). Major metabolites of TCE (TCE-epoxide, dichloroethylene, and vinyl chloride) and perchloroethylene (PCE) also cause serious groundwater contamination problems (Westrick et al., 1984). These chemicals are suspected carcinogens and generally tend to resist biodegradation in the environment (Infante and Tsongas, 1982; Love and Eilers, 1982).

Microorganisms capable of utilizing TCE as a sole source of carbon and energy for growth have not been reported. However, TCE can be degraded by bacterial cometabolism under anaerobic (Fig. 5) and aerobic (Fig. 6) conditions. Under anaerobic conditions, TCE can be transformed as a non-growth-supporting electron acceptor. As a result, TCE can be sequentially dechlorinated, producing less-chlorinated metabolites (Vogel and McCarty, 1985). However, under anaerobic conditions, dechlorination is very slow, leading to accumulation of a more toxic metabolite, vinyl chloride. Under aerobic conditions, TCE can be biologically oxidized by broad specific monooxygenases and dioxygenases, which are normally produced to initiate the oxidation of growth-supporting substrates such as methane, ammonia, phenol and toluene (Ensley, 1991; Little et al., 1988). Although all oxygenases have the capacity to oxidize their primary substrates, only a handful of oxygenases have

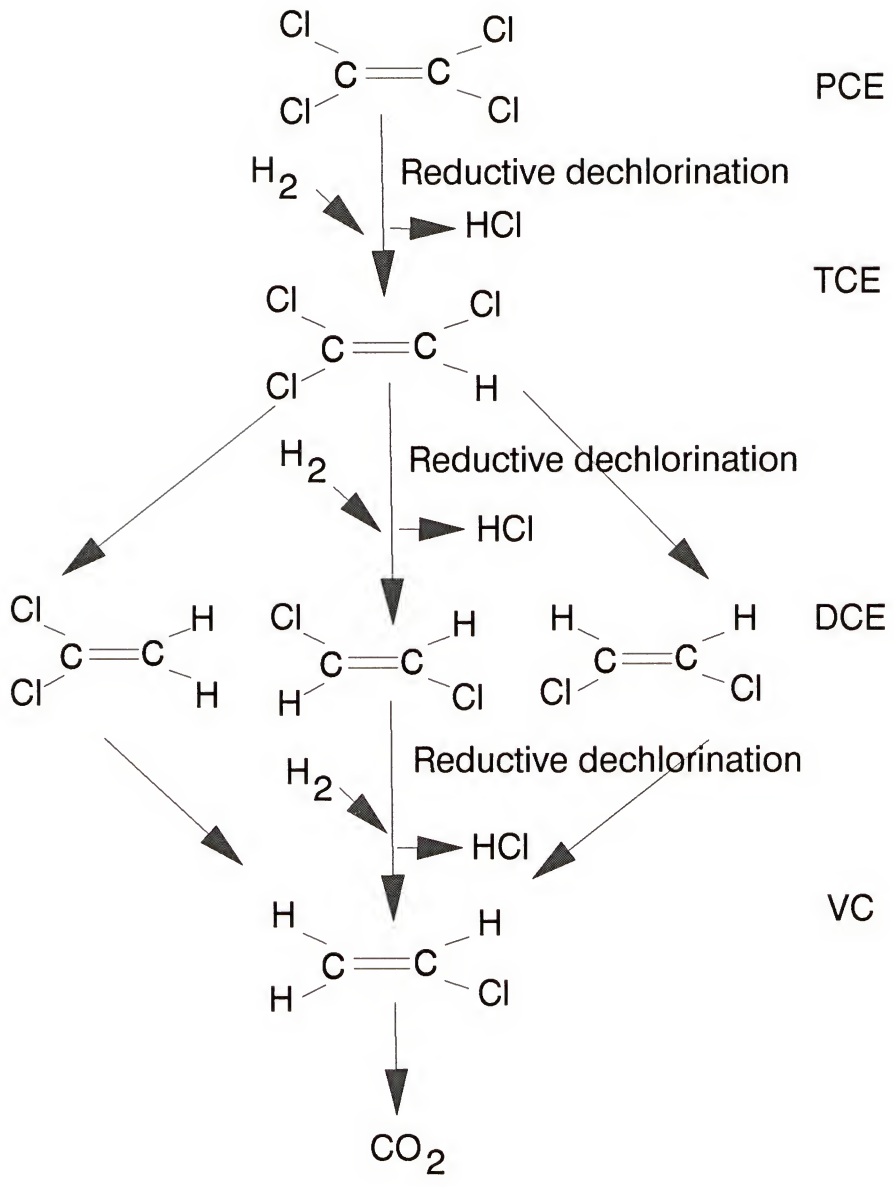


Figure 5. Proposed anaerobic degradation pathways of TCE (Vogel and McCarty, 1985).

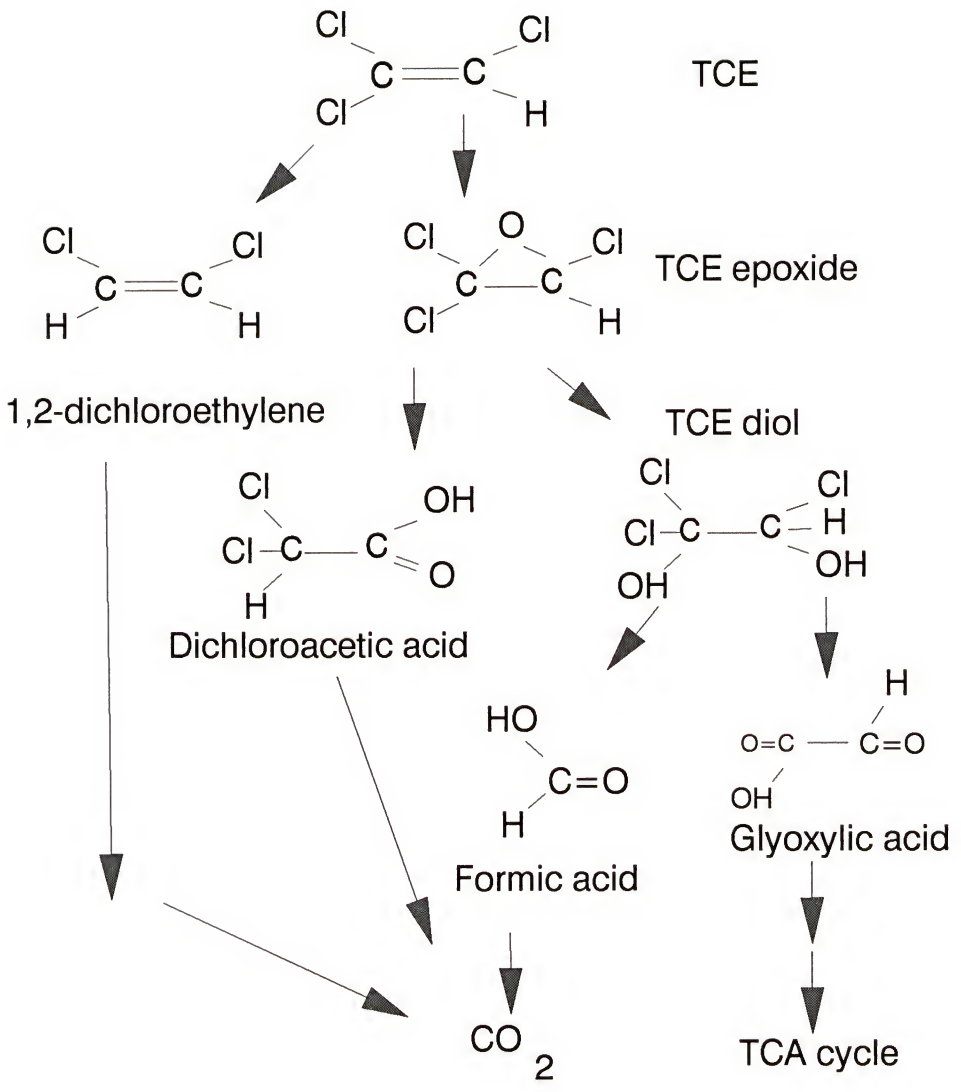


Figure 6. Proposed aerobic degradation pathways of TCE (Little et al., 1988).

the capacity to oxidize TCE or other short-chain, halogenated, aliphatic hydrocarbons.

Methanotrophic bacteria are aerobic microorganisms and are ubiquitously present in oxic soil and aquatic environments. These bacteria possess unique methane monooxygenase (MMO) enzyme system which enables them to utilize methane as a sole carbon and energy source (Dalton et al., 1984). There are two types of MMO, soluble methane monooxygenases (sMMO) and particulated (membrane-associated) MMO (pMMO), produced by some methanotrophs that have the capacity to degrade TCE, with sMMO being the major factor (DiSpirito et al.,1992). Additionally, some sMMO can degrade a wide range of carbon substrates (Dalton, 1992) which are economically and environmentally important organic compounds (Higgins et al., 1980).

Wilson and Wilson (1985) were the first to demonstrate that TCE could be mineralized to CO_2 and H_2O in methanotrophic soil. Fogel et al.(1986) reported that TCE was also mineralized to CO_2, H_2O, and Cl^- by methane-oxidizing bacteria in a liquid medium. Arciero et al.(1989) reported that the ammonia-oxidizing bacterium *Nitrosomonas europaea* cometabolically degraded TCE in the presence of ammonia. Ammonia-oxidizing bacteria oxidize ammonia to nitrite for growth. They produce ammonia monooxygenases (AMO), membrane-bound enzymes, that catalyze the oxidation of ammonia to hydroxylamine (Hyman and Wood, 1983).

Ammonia monooxygenases are also capable of cooxidizing a variety of organic compounds including methane (Hyman and Wood, 1983), methanol (Vosey and Wood, 1987), ethylene (Hyman and Wood, 1984), propylene (Drozd, 1980), benzene and phenol (Hyman et al., 1985), cyclohexane (Drozd, 1980), bromocarbons (Hyman and Wood, 1984), and carbon monoxide (Jones and Morita, 1983).

Methyl Bromide

Methyl bromide (MeBr) is presently the most important fumigant commercially available (Noling and Becker, 1994). At ≥4°C, MeBr is a gas (Ou et al., 1997). This chemical is extensively used for the control of nematodes, soil-borne fungi, and weeds (Fergusion and Padula, 1994). This chemical is also used as a space fumigant for commodities, for structural pest control, and for quarantine and regulatory purposes (Ou et al., 1997).

Despite its beneficial uses in agriculture, use of MeBr will be suspended in the year 2001 (Noling and Becker, 1994). It is a potent depleter of the stratospheric ozone layer (Watson et al., 1992), due to its release of bromine atoms into the atmosphere. Bromide ion is as much as 100-fold more efficient at scavenging ozone than Cl ion (Prather and Watson, 1990). It is currently believed that anthropogenic MeBr sources, such as from agricultural soil fumigation, account for about one-third of the annual flux of 200×10^6 kg, with the rest derived from natural sources

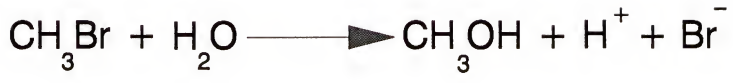
such as the oceans and the burning of forests (Butler, 1994; Khalil et al., 1993).

In water, MeBr is chemically hydrolyzed to methanol, and may exchange with other halides (Elliot and Rowland, 1993). In soil, MeBr undergoes both chemical and biological degradation, which include chemical hydrolysis (Gentile et al., 1989), methylation to soil organic matter (Gan et al., 1994) and microbial oxidation (Oremland et al., 1994; Ou et al., 1997).

Similar to TCE, MMOs are involved in the oxidation of MeBr to formaldehyde and a bromide ion. Some ammonia-oxidizing bacteria and methane-oxidizing bacteria in the presence of their primary substrates, ammonia and methane, have been shown to have the capacity to oxidize MeBr (Meyers, 1980; Oremland et al., 1994; Rasche et al., 1990). Ou et al. (1997) reported that the degradation of MeBr in soil can be stimulated by increase in the activity of soil nitrifiers through the application of ammonium fertilizers. In this case, the major degradation pathway of MeBr is the oxidation of MeBr to formaldehyde (Fig. 7).

Chemical Degradation

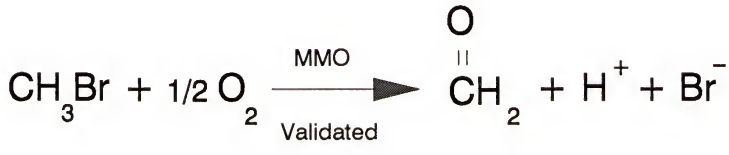
i. Hydrolysis



ii. Methylation

Biological Degradation

i. Oxidation



ii. Hydrolysis

Figure 7. Proposed degradation pathways of MeBr in soils (Ou et al., 1997).

Degradation and Metabolic Pathways of 1,3-Dichloropropene (1,3-D) in Soil and by Microorganisms

Chemical and Physical Properties of Cis- and Trans-1,3-D

1,3-Dichloropropene (1,3-D) is the active ingredient of two commercial fumigants, Telone II (94%), and Telone C-17 (78%), marketed by DowElanco (DowElanco, 1996). These fumigants are applied before crops are planted. 1,3-Dichloropropene consists of two isomers, cis- and trans-1,3-D (Fig. 4). This chemical, in commercial formulations, is generally composed of an equal mixture of the two isomers. The chemical is a colorless to straw-colored liquid, with sweet penetrating ordor (Table 1).

Degradation and Metabolism of 1,3-D in Soils

Cis- and trans-1,3-D in soils can be volatilized into the atmosphere through diffusion from soil pores to the soil surface. Furthermore, their high water solubility makes the movement of 1,3-D into the groundwater possible (Leistra et al., 1991; Van der Pas and Leistra, 1987).

The first step in the degradation of cis- and trans-1,3-D in soils involves hydrolysis to the corresponding cis- and trans-3-chloroallyl alcohol (CAA). The hydrolysis is considered to be chemical, rather than biological (Roberts and Stoydin, 1976). Cis- and trans-3-CAA are then oxidized to the corresponding 3-chloroacrylic acid (CCA). Eventually, the two 3-CCA isomers are mineralized to CO_2, H_2O, and Cl^- (Ou, 1989; Roberts and Stoydin, 1976).

Table 1. Chemical and physical properties of cis- and trans-1,3-D.

Property	Cis-1,3-D	Trans-1,3-D
Chemical formula	$CHCl=CHCH_2Cl$	
Molecular weight	110.97	
Physical state	Liquid	
Vapour pressure (mm Hg at 25°C)	43	34
Color	Colorless or straw-colored	
Odor	Sweet, penetrating	
Density (at 20°C)	1.205	1.219
Boiling point (°C)	104.1°C	112.6°C
Melting point (°C)	<-50°C	<-50°C
Water solubility (μg/mL)	2180	2320
Partition coefficient (Log Kow)	2.09	2.04
Henry's law constant (atm/gmol)	$1.8x10^{-3}$	$1.05x10^{-3}$

(DowElanco, 1996; Yang, 1986).

Castro and Belser (1966) were the first to demonstrate that cis- and trans-1,3-D in soil suspensions were hydrolyzed to cis- and trans-3-CAA. They also found that the degradation of 1,3-D in soil suspensions was faster than in buffered water. They did not know whether biological hydrolysis was also involved in the degradation of 1,3-D in soil.

The degradation pathways and metabolites of cis- and trans-1,3-D in two British soils were extensively investigated by Roberts and Stoydin (1976)(Fig. 8). They used ^{14}C-labeled cis- and trans-1,3-D for determination of metabolites and mass balance, and concluded that 3-CAA was the major metabolite and 3-CCA the minor metabolite. They proposed that cis- and trans-1,3-D in soils were hydrolyzed to the corresponding cis- and trans-3-CAA forms, which, in turn, were oxidized to cis- and trans-3-CCA. 3-Chloroacrylic acid was then either converted to bound residues or further degraded. Even though their mass-balance results for ^{14}C-cis-1,3-D and ^{14}C-trans-1,3-D were good, ranging from 70-102%, they did not determine $^{14}CO_2$ evolution from these soils. As a result, the mass-balance results were not complete. Ou (1989) subsequently demonstrated that soil indeed had the capacity to mineralize ^{14}C-1,3-D (a mixture of cis- and trans-1,3-D), with mineralization of 1,3-D being a microbial process. Ou also found that, due to its volatile nature, mass balance was poor, ranging from 50 to 70%.

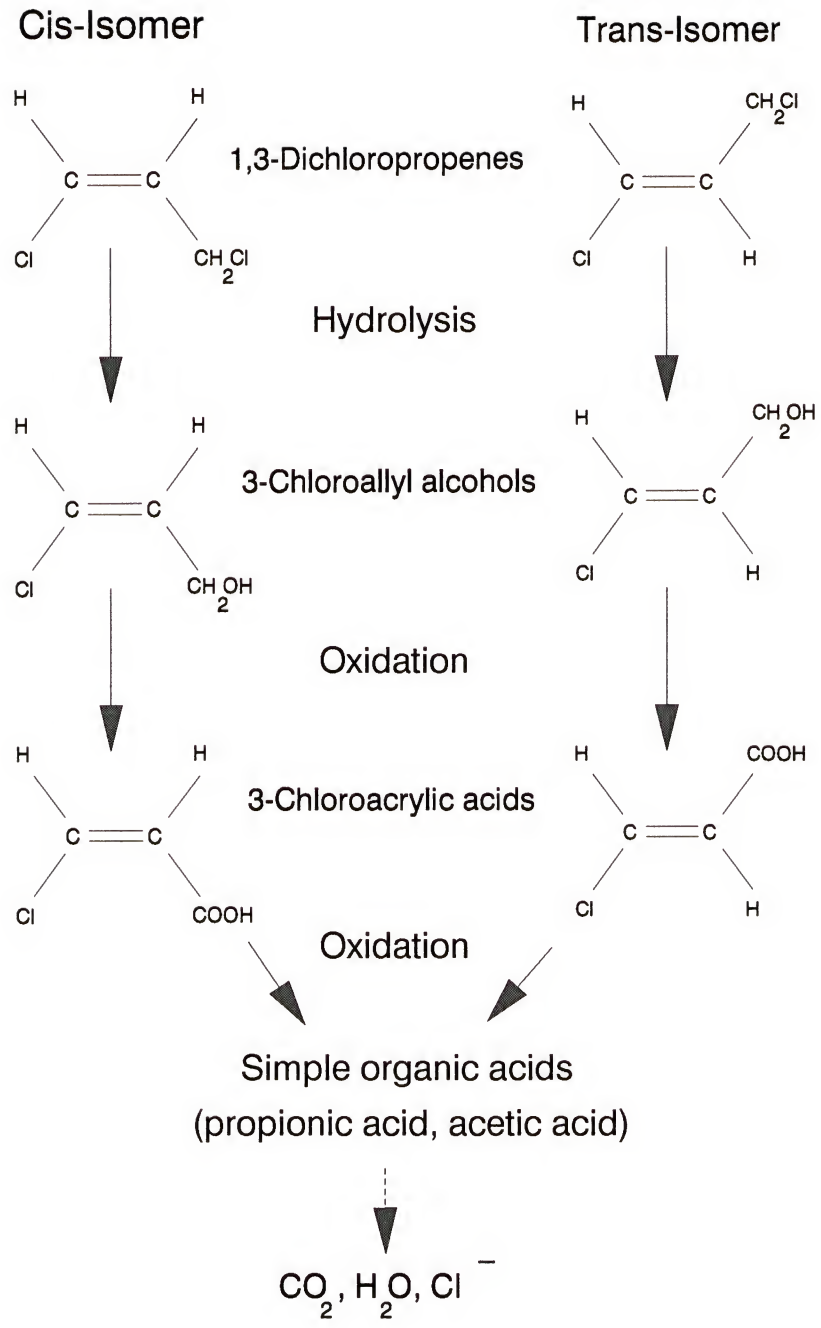


Figure 8. Proposed degradation pathways of cis- and trans-1,3-D in soils (Roberts and Stoydin, 1976).

The majority of the published information on 1,3-D degradation in soils has emphasized the disappearance rates for cis-1,3-D and trans-1,3-D, for a mixture of the two isomers. Most studies were conducted in the laboratory in closed systems, and generally an equal mixture of cis- and trans-1,3-D was applied to soils. Despite their volatility, no efforts were made to determine mass balance. Van Dijk (1980) showed that the disappearance rates of the two isomers were dependent on soil type, temperature, and moisture content, with both isomers in the same soil disappearing at similar rates. No clear correlations were found with soil organic matter content, clay content, and soil pH.

Disappearance rates for cis- and trans-1,3-D in various soils in the Netherlands at 10, 15, and 20°C were reported to be highly variable (Leistra et al., 1991; Van der Pas and Leistra, 1987; Van Dijk, 1974, 1980). Half-life values for cis-1,3-D in these soils at 10°C, 15°C, and 20°C, were 16 to 46, 7 to 33, and 3 to 19 days, respectively. The half-life values for trans-1,3-D at the corresponding temperatures were 17 to 47, 4 to 32, and 3 to 15 days, respectively. This suggests that the half-life values of the two isomers were not different, and that an increase in temperature resulted in a decrease in half-life values for the two isomers. It appears that the large variation of the half-life values

was due to differing soil type, climate (including temperature) and volatilization loss. Information on prior histories of field applications of 1,3-D to these soils was not provided. It was possible that enhanced degradation of 1,3-D in some of the soils might have occurred because of repeated field applications of the chemical.

The effect of soil-water content on the disappearance of the two isomers in soils was not as pronounced as that of temperature (Van Dijk, 1980). The half-life values of cis-1,3-D in soil at field capacity and in water-saturated soil were 7 to 16 and 6 to 14 days, respectively. These values were not significantly different, but the half-life values in air-dry soils were much larger (27 to 35 days).

Chemical hydrolysis rate of 1,3-D in sterile buffered water increased with an increase in temperature (McCall, 1987). However, hydrolysis rate was independent of pH at each temperature. The measured half-life values for hydrolysis in the buffered water were 3.1 (±0.1), 11.3 (±0.5), and 51 (±2.3) days at 30, 20, and 10 °C, respectively. 3-CAA was the hydrolysis product. The analytical technique employed for the determination of 1,3-D, an HPLC-radiotracer, was not able to separate the two isomers. Therefore, the hydrolysis rate represented a mixture of the two isomers. As a result, the hydrolysis rates of the two isomers were considered to be the same.

Since 1,3-D is volatile, the true degradation rates of cis- and trans-1,3-D in soil under field conditions is difficult to determine. Even under laboratory conditions, a number of careful measurements have to be taken so that reliable degradation rates can be obtained. These include use of only tightly closed systems, including keeping all glass or stainless steel containers (which contain soil samples and are equipped with Teflon-lined caps) cold prior to introducing cold organic solvent for extraction, and avoiding frequent opening and closing of the containers. Use of ^{14}C-1,3-D is highly desirable so that mass balance, mineralization, and bound residues can be determined. Ou (1989) found that, after 28 days of incubation, ^{14}C-1,3-D in soil was distributed into $^{14}CO_2$, water-soluble metabolites, bound residues, and possibly microbial mass. Since the containers for ^{14}C-1,3-D-treated soil samples had to be opened and closed a number of times during the incubation period, some of the ^{14}C was undoubtedly lost due to volatilization. As a result, ^{14}C recovery was low, only 50 to 70%. In a more careful study taking precautions mentioned above, Ou et al. (1995) were able to recover as much as 92-96% of the applied 1,3-D, with typical recovery in the range 72-75%.

Some short-chain halogenated hydrocarbons are suspected or proven carcinogens, and frequent groundwater contaminants. Efforts have been made to detect 1,3-D

residues in groundwater underneath agricultural fields where 1,3-D was applied, but no 1,3-D residues were detected in 54 well-water samples from California (Maddy et al., 1982). On the other hand, because of the use of 1,3-D for flower production and the peculiar geological conditions in the Netherlands, downward movement of 1,3-D into groundwater has been extensively studied there (Leistra et al., 1991; Van der Pas and Leistra, 1987). Low levels of 1,3-D residues were occasionally detected in the shallow groundwater samples collected beneath a commercial flower-production field (Van der Pas and Leistra, 1987). It should be noted that groundwater tables in California are deep, whereas groundwater tables in the Netherlands are shallow. Since 1,3-D in water and soil is rapidly hydrolyzed, it is not surprising that no 1,3-D residues were detected in California groundwater samples. Further research should be directed to detection of 3-CAA residues in groundwater and determination of the corresponding toxicity of 3-CAA to animals and humans.

Enhanced Degradation of 1,3-D in Soils

Two studies on enhanced degradation of 1,3-D in soils (Lebbink et al., 1989; Smelt et al., 1989) were reported at nearly concurrently, and both involved soils from the Netherlands. Smelt et al. (1989) reported that enhanced degradation of 1,3-D might have occurred in some loamy soils collected from a number of fields with or without previous

exposure to 1,3-D. Lebbink et al. (1989) reported accelerated degradation of 1,3-D in soil suspensions using soil collected from a field site that had been treated with 1,3-D annually for 12 years. All soil suspensions were supplemented with organic and inorganic nutrients. They also found that nematicidal efficacy progressively decreased with an increase in the number of annual field applications of 1,3-D. In both studies, individual disappearance rates of cis- and trans-1,3-D were not investigated. Furthermore, untreated sites were not established. As a result, no untreated soil samples were used for determination of their capacity to degrade 1,3-D. Without the use of control soils, whether or not enhanced degradation occurs in the treated soils cannot be validated. Thus, it is not clear if repeated applications of 1,3-D to the same field soils caused enhanced degradation of the chemical.

Extensive repeated applications of cis-1,3-D to microplots resulted in accelerated degradation of the isomer (Verhagen et al., 1995). Some microplots were repeatedly treated with cis-1,3-D, up to six times over a period of twelve months. Degradation of cis-1,3-D in the treated microplots was faster than in the untreated microplots. It is not known whether enhanced degradation of trans-1,3-D also occurred in the cis-1,3-D treated soils.

Ou et al. (1995) were the first to demonstrate true enhanced degradation of cis- and trans-1,3-D in a Florida sandy soil that had been repeatedly treated with 1,3-D. Surface (0-15 cm) and subsurface (15-30 cm) soil samples were collected from an experimental site near Gainesville, FL. This site had been treated with 1,3-D, six times over a span of 12 years, and had been planted continuously with either peanut or tomato. Unlike the previous two studies (Lebbink et al., 1989; Smelt et al, 1989), Ou et al. (1995) established a control site which had never been exposed to 1,3-D. Cis- and trans-1,3-D in treated surface (0-15 cm) and shallow subsurface (15-30 cm) soils disappeared more rapidly than in the corresponding control soils. Furthermore, trans-1,3-D in the treated soils disappeared faster than cis-1,3-D, whereas the degradation rates of the two isomers in the control soil were not different from each other. In addition, little or no trans-3-chloroallyl alcohol was detected in treated soils, but large amounts of cis-CAA were formed in treated soils. Initial mineralization rates of ^{14}C-1,3-D in the treated soils were greater than in the untreated soils.

Yon et al. (1991) reported rapid degradation of ^{14}C-1,3-D in soil/water slurries collected from a ditch (near a 1,3-D production plant) which was known to be a habitat of a variey of microbial populations and highly biologically active. They showed that ^{14}C-1,3-D in the soil/water

slurries was rapidly transformed to 3-chloroacrylic acids and other unknown metabolites, whereas rapid evaporation occurred from water without soil. The half-life value of 1,3-D was less than 5 hours in the water systems. Thus, they assumed that the transformation mechanisms involved in the metabolic pathways in soil/water slurries were biological, rather than chemical, due to the faster transformation rates.

Degradation of 1,3-D by Microorganisms

Microorganisms appear to be responsible for enhanced degradation of pesticides (Racke and Coats, 1990). However, similar to other short-chain halogenated hydrocarbons, microorganisms capable of utilizing 1,3-D as a sole source of carbon for growth and energy have not been isolated from Florida sandy soils. Ou (1989) enriched a mixed bacterial culture from soil at a 1,3-D treated site in Florida, using glucose as a second carbon source. The mixed culture had a good capacity to mineralize 1,3-D, with up to 44% of the ^{14}C-1,3-D being mineralized by the mixed culture in 28 days. Without the supplement of a readily degradable carbon source such as glucose, the mixed culture did not grow and did not appear to mineralize 1,3-D. A number of axenic bacterial cultures that had limited capacity to mineralize 1,3-D were also isolated from soil. Therefore, 1,3-D in soil is mainly biologically degraded through cometabolic processes (Ou, 1989).

Two studies have reported successful isolation of axenic bacterial cultures capable of degrading 1,3-D from Dutch soils (Lebbink et al., 1989; Verhagen et al., 1995). Lebbink et al. (1989) isolated a strain of a *Pseudomonas* sp. from a soil that had been continuously treated with 1,3-D for 12 years. They claimed that the isolate was able to utilize 1,3-D as a sole source of carbon for its energy and growth. However, it is questionable that this isolate indeed could utilize 1,3-D as a sole source of carbon for growth. The isolate was maintained in a rich liquid medium containing several biodegradable organic chemicals. The *Pseudomonas* sp. degraded trans-1,3-D more rapidly than cis-1,3-D. This supported the findings of Ou et al. (1995) that trans-1,3-D in enhanced soils was degraded more rapidly than cis-1,3-D. Verhagen et al. (1995) isolated six axenic bacterial cultures capable of utilizing cis-1,3-D as a sole source of carbon and energy from soil samples collected from microplots that had been intensively treated with cis-1,3-D for a span of one year. Again, it was questionable that these isolates were truly capable of solely utilizing cis-1,3-D for growth, because yeast extract was used for maintaining these cultures. The isolates harbored plasmids and Verhagen et al. (1995) suggested that the plasmids were responsible for the degradation of cis-1,3-D. It is not known whether these isolates also had the capacity to degrade trans-1,3-D.

3-Chloroallyl alcohol in soils was also found to be rapidly degraded (Leistra et al., 1991; Van Dijk, 1980). A strain of *Pseudomonas* sp. isolated from a California soil had the capacity to utilize 3-CAA as a sole source of carbon for growth (Belser and Castro, 1971). Van Waarde et al. (1993) also isolated three strains of *Pseudomonas* sp. from a Dutch soil capable of utilizing 2-chloroallyl alcohol, an analogous chemical of 3-chloroallyl alcohol, as a sole source of carbon for growth. Thus, it appears that chloroallyl alcohols can serve as a sole source of carbon for a number of soil microorganisms, especially *Pseudomonas* sp.

RESEARCH OBJECTIVES

1,3-Dichloropropene (1,3-D) is a soil fumigant which is used for the control of plant parasitic nematodes and other soil-borne pests. This fumigant is extensively used in Florida and California in the U.S.A., and in the Netherlands. 1,3-D may be considered to be a viable alternative to methyl bromide. Due to suspected depletion of the stratospheric ozone layer, the use of methyl bromide in agriculture will be suspended in the year 2001. 1,3-D consists of two isomers, cis- and trans-1,3-D. The two isomers are short-chain halogenated aliphatic hydrocarbon compounds. Microbial degradation patterns of the two isomers may be similar to those for some short-chain halogenated aliphatic compounds such as methyl bromide and trichloroethylene (TCE).

Repeated field applications of 1,3-D may cause differential enhanced degradation of the two isomers and increase the degree of the enhancement of the two isomers after additional annual applications. Consequently, the enhanced degradation of the fumigant may result in poor control of nematodes, and therefore, may lead to an economic loss for the agricultural industries in Florida.

Little information is available about the extent, duration, and microbial basis for the differential enhanced degradation of cis- and trans-1,3-D. Therefore, the following are the four major research objectives of my dissertation research:

1. To determine degradation rates of cis- and trans-1,3-D in surface and subsurface soils from an enhanced site after three consecutive annual applications of 1,3-D.
2. To determine duration of the enhanced degradation.
3. To confirm an earlier finding that cis- and trans-1,3-D in enhanced soil were differentially degraded, with the trans isomer being degraded faster than the cis isomer.
4. To isolate microorganisms capable of degrading cis- and trans-1,3-D from enhanced soil.

MATERIALS AND METHODS

Materials

Site Description

The Green Acres Farm where soil samples were collected is located 12 miles west of the campus of the University of Florida. As of July, 1997, a part of this farm had been treated with Telone II annually or biannually at least 9 times over the past 15 years at a rate of 1.04 to 1.95 kilograms per hectare. Since 1994, the Green Acres site was treated with Telone II annually either in late spring or early summer at a rate of 1.56 or 1.95 kg/ha (Table 2), and was planted to peanut or tomato. A nearby site at this farm that had never been treated with 1,3-D was established as a control. After the 1995 annual application, a small area of the treated site was established and has not been treated with 1,3-D since (Table 2). Soils from the treated and control sites were classified as Arredondo fine sand (a loamy, siliceous, hyperthermic Grossarenic Paleudult).

Soil Sampling

Soil samples at 15-cm increments to the 45-cm depth were collected from the treated and control sites, using a 10-cm diameter bucket auger. Soil samples were collected from the treated and control sites in 3 consecutive

Table 2. Application dates and rates of 1,3-D applied to the treated site at the Green Acres Farm from 1994 to 1997.

Application Date	Application Rate (kg/ha)
April 27, 1994	1.56
June 29, 1995	1.56
April 23, 1996	1.56
July 29, 1997	1.95

years (1995, 1996, and 1997), 0.7, 5, 10, and 24 months after the 1995 annual field application of 1,3-D; 0.7, 6, and 10 months after the 1996 annual application; and 0.7 month after the 1997 annual application. Each soil sample was a composite of 3 cores from the same depth. All samples were placed in double plastic bags, transported to the laboratory, and stored in the dark at 4°C. All samples were used within 3 months after collection.

Selected properties of the soil samples are given in Table 3.

Chemicals

Telone II (94% commercial grade), analytical grade cis- and trans-1,3-D (98-99% purity), and analytical grade cis- and trans-3-CAA (98% purity) were provided by DowElanco Corporation (Indianapolis, IN). All other chemicals were pesticide-grade, analytical grade, or the highest grade commercially available.

Table 3. Selected properties of soil samples collected from the Green Acres site.

Soil	Depth	pH (1:1 = soil: D.I.H_2O)	Organic C	Soil-water content	Particle size analysis		
					Sand	Silt	Clay
	cm			g kg^{-1}			
Treated	0-15	6.0	6.7	61	918	79	3
	15-30	6.0	6.2	68	943	54	3
	30-45	6.3	1.9	67	923	74	3
Control	0-15	6.4	5.2	79	934	63	3
	15-30	6.1	4.9	95	901	95	4
	30-45	6.0	1.9	66	782	211	7

Culture Media

Two basic media, basal mineral-trace mineral media (BMTMM) and soil extract, were used. Other culture media used in this study were modified from the two basic media. Two types of soil extracts were used as well. They were soil extract (Ou, 1991) and soil leachate. The basal mineral medium was composed of K_2HPO_4, 4.8 g; KH_2PO_4, 1.2 g; NH_4NO_3, 1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2$, 0.04 g; $Fe_2(SO_4)_3 \cdot 7H_2O$, 0.001 g; and deionized H_2O, 1 L. The pH of the medium was adjusted to 7.2. Stock trace minerals were made up of $MnSO_4 \cdot 6H_2O$, 2 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; $ZnSO_4 \cdot 6H_2O$, 2 mg; H_3BO_3, 0.3 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.4 mg; $CoCl_2 \cdot 2H_2O$, 0.4 mg; and deionized H_2O, 1 L (Ou and Thomas, 1994). The stock trace minerals were sterilized by filtration. When supplemented, 500 μL of the stock trace minerals was added to 50 mL of sterile MMA.

Preparation of Soil Extract

Soil extract was prepared by the procedure of Ou (1991). One kg of field-treated soil and 1 liter of deionized water were added to a 2 L Erlenmyer flask, and the flask was autoclaved at 121°C for 1 hour. After soil particles had settled overnight, the supernatant was carefully poured into 500 mL plastic centrifuge bottles, and the bottles were centrifuged at a speed of 10,000 rpm. Nalgene microfilters (<0.22 μm) were used for filtration and sterilization of the centrifuged soil extract, with the extract being kept in a refrigerator (4°C) until use.

Preparation of Soil Leachate

Five hundred grams of field-treated soil and 250 mL of deionized water (2 to 1 ratio) were added to a 1 L Erlenmyer flask and then shaken for 10 minutes. Soil particles were allowed to settle overnight, and the supernatant was transferred to 500 mL plastic centrifuge bottles. These bottles were then centrifuged at a speed of 5,000 rpm for 20 minutes. Nalgene microfilters (<0.22 μm) were used for filtration and sterilization of the extract to keep heat-labile soil nutrients intact. The filtered extract was maintained at room temperature (25°C) until use.

Preparation of L-Plates and L-Broth

L-agar plates - which are composed of 200 mL of deionized water, 2 g of tryptone, 1 g of yeast extract, 1 g of NaCl, and 4 g of agar - were used for isolation of axenic cultures from mixed cultures and for checking purity of axenic cultures. L-broth was the same as for the L-plates, except that agar was not added.

Preparation of Yeast Extract, Tryptone and Tryptophan Solutions

One percent solutions of yeast extract, tryptone, or tryptophan in deionized water were prepared, and were sterilized by autoclaving.

Methods

Soil Treatment

Ten grams of each soil (oven-dry weight basis) were placed in a 40 mL glass centrifuge tube equipped with a

Teflon-lined screw cap. Using a glass micro syringe with scales of 0.1 μL, three tenth μL of an equal mixture of analytical grade cis- and trans-1,3-D, equivalent to 20 μg g^{-1} of each isomer, was added to the top of each soil, and the tube was immediately closed tightly. After the centrifuge tubes were shaken on a reciprocal shaker at 500 strokes per minute for 10 minutes, all tubes were incubated in the dark at 28°C. At desired time intervals, two tubes from each treatment were removed for solvent extraction.

Methane Treatment

Sixty mL of methane was introduced weekly with a 100 mL syringe into a 1 L glass bottle equipped with a Teflon-lined cap, which contained 500 g of soil. Deionized H_2O was added periodically to compensate for the loss of water. This treatment lasted for 1 month.

Ammonium Sulfate Treatment

Ammonium sulfate at a rate of 150 μg g^{-1} was added twice to 500 g of soil at an interval of 4 weeks, and each pH was adjusted with calcium oxide to between 7.2 and 7.5. The treatment was continued for 2 months.

Soil Autoclaving

One hundred grams of treated surface (0-15 cm) and shallow subsurface (15-30 cm) soil were autoclaved at 121°C for 60 minutes.

Soil Extraction

At predetermined intervals, two tubes from each treatment (10 g of soil) were removed from the incubator and placed in an ultra low freezer (-32°C) for 1 hour. Afterward, twenty mL of cold acetone was added to each of the tubes, which were immediately closed. The centrifuge tubes were shaken on a reciprocal shaker at 500 rpm for 1 hour. After shaking, the tubes then were centrifuged at 1500 rpm for 15 minutes. One mL of the supernatant solution was transferred to a glass GC vial for gas chromatography (GC) analysis of cis- and trans-1,3-D, and of cis- and trans-3-CAA.

Enrichment and Isolation of Cis- and Trans-1,3-D Degrading Microorganisms

The basal mineral-trace mineral medium (BMTMM), when containing a small amount of enhanced soil (0.01 g/mL), was found to have the capacity to degrade cis- and trans-1,3-D. Small amounts of this suspension then were streaked on L-agar plates. Individual colonies did not have the capacity to degrade cis- and trans-1,3-D when grown in BMTMM supplemented with soil leachate (0.04 mL/mL), but a mixed bacterial cuture from a mixed bacterial colony grown in the same medium was found to have the capacity to degrade the two isomers. Table 4 shows various culture media containing various biodegradable second carbon sources that were used for screening a second carbon substrate capable of stimulating 1,3-D degradation.

Fifty mL of each test medium (Table 4) was placed in a 250 ml glass flask with a Teflon-lined cap. To prevent

Table 4. Various test media containing various second carbon substrates were used to screen for a second carbon substrate that had the capacity to stimulate the degradation of cis- and trans-1,3-D by the mixed culture.

Medium
Basal mineral-trace mineral media (BMTMM) + enhanced soil (0.01 g/mL)
BMTMM + autoclaved enhanced soil (0.01 g/mL)
BMTMM + soil leachate (0.04 mL/mL)
BMTMM + glucose (200 μg/mL)
BMTMM + yeast extract (200 μg/mL)
L-broth
BMTMM + L-broth (5 μL/mL)
BMTMM + tryptone (200 μg/mL)
BMTMM + tryptophan (200 μg/mL)

leaks, the top of each flask was wrapped with Teflon tape. After cis- and trans-1,3-D (25 μg/mL each) were added, the flask was immediately closed with the cap. To prevent photodecomposition, all flasks were wrapped with aluminum foil, and incubated on a rotary shaker at 28°C. At desired time intervals, 0.5 mL of the culture fluids was removed and transferred to a glass culture tube for hexane extraction.

Culture Extraction

Ten mL of cold hexane were added to a culture tube containing 0.5 mL of a culture fluid, and the tube was

shaken on a reciprocal shaker at 500 strokes per minute for 10 minutes. After shaking, one mL of the hexane extract was transferred to a GC vial for GC analysis of cis- and trans-1,3-D.

GC Analysis

The analytical procedures for quantification of cis- and trans-1,3-D, and cis- and trans-3-CAA, were similar to the procedures previously described by Ou et al. (1995). Cis- and trans-1,3-dichloropropene and their corresponding hydrolysis products, cis- and trans-3-CAA, were quantified by a Perkin Elmer Autosystem GC equipped with an autosampler, ^{63}Ni electron capture detector (ECD), split-splitless injector, Turbochrom 4 software, and a 486 computer. The GC parameters and operational conditions were as follows: column, 30 m x 0.25 mm i.d., RTX-624 coated with 3 μm film thickness; flow rates for carrier gas (He) and make-up gas (99.5% N_2 and 0.5% CH_4), 5 mL/minute and 30 mL/minute, respectively; injector temperature, 150°C; detector temperature, 375°C; oven temperature, 50°C for the first minute, ramp at 40°C/minute, and hold at 120°C for 16 minutes; split valve, off for the first 1 minute for analysis of 1,3-D or the first 1.5 minutes for analysis of 3-CAA; and injection volume of 1 μL for 1,3-D and 3-CAA. Under these conditions, the retention times for cis- and trans-1,3-D and cis- and trans-3-CAA were 4.7, 5.0, 5.4, and 5.6 minutes, respectively. Limits of detection (Hubaux and

Vos, 1970 and Ott, 1977) for cis- and trans-1,3-D and cis- and trans-3-CAA were 0.26, 0.28, and 0.66, and 0.54 μg/mL, respectively.

Mathematical Equations Used for Calculation of Half-Life Values ($t_{1/2}$)

Parent chemical disappearance rate coefficients (K_1) of cis- and trans-1,3-D in treated and untreated (control) soils were expressed by the following first-order kinetics equation:

$$C(t) = C_o * e^{-K*t} \quad (1)$$

where

K = parent chemical disappearance rate coefficients of cis- and trans-1,3-D

C_o = initial concentration (μg/g)

C(t) = concentration at time t (μg/g).

Half-life values were calculated using the following equation:

$$t_{1/2} = Ln\ (1/2)/K \quad (2)$$

where

$t_{1/2}$ = half-life (days)

K = first-order rate coefficient ($days^{-1}$).

RESULTS

For comparison of the degradation rates of cis- and trans-1,3-D in treated and control (untreated) soils, control soils were collected 3 weeks after annual application of 1,3-D in June or July of 1995, 1996, and 1997. Therefore, three sets of control soils were used for determination of the degradation rates of cis- and trans-1,3-D.

Degradation rates of cis- and trans-1,3-D in the control soil samples collected 3 weeks after the 1995 annual application were used for comparison with degradation rates of cis- and trans-1,3-D in treated soil samples collected 0.7 month (3 weeks) and 6 months after the 1995 annual application (Figs. 9 and 10). Degradation rates of cis- and trans-1,3-D in the control soil samples collected 3 weeks after the 1996 annual application were used for comparison with the degradation rates of the two isomers in treated soil samples 10 months after the 1995 annual application (Fig. 11) and 0.7 month and 5 months after the 1996 annual application (Figs. 13 and 14). Degradation rates of cis- and trans-1,3-D in the control soil samples collected 3 weeks after the 1997 anual application with 1,3-D were used for comparison with degradation rates of the two isomers in

treated soil samples collected 24 months (2 years) after the 1995 annual application (Fig. 12), 10 months after the 1996 annual application (Fig. 15), 0.7 month (3 weeks) after the 1997 annual application (Fig. 16) and 0.7 month after the 1997 reapplication to a part of the treated site that had not been treated with 1,3-D for 2 years (Fig. 17).

Degradation of Cis- and Trans-1,3-D in Soil Treated with 1,3-D in 1995

Three Weeks after Annual Application

Trans-1,3-dichloropropene in the treated surface soil (0-15 cm depth) collected 3 weeks after annual application was degraded more rapidly during the first 5 days of incubation than its counterpart, cis-1,3-D (Fig. 9A). After 5 days, cis-1,3-D was more rapidly degraded. As a result, after 7 days, both cis- and trans-1,3-D were either nearly completely or completely degraded and, after 10 days, neither of the isomers were detected in the treated soil. Similar degradation patterns were observed in the treated shallow subsurface soil (15-30 cm depth) (Fig. 9B) with the exception that, during the first 3 days, cis-1,3-D was degraded faster than in the surface soil. As a result, the amount of cis-1,3-D remaining in the shallow subsoil was only slightly larger than for trans-1,3-D. Similar to the surface soil, after 7 days, negligible amounts of the two isomers were detected in each case. In contrast to the two upper layers of soil, trans-1,3-D in the subsurface layer of 30-45 cm depth during the first 3 days of incubation was

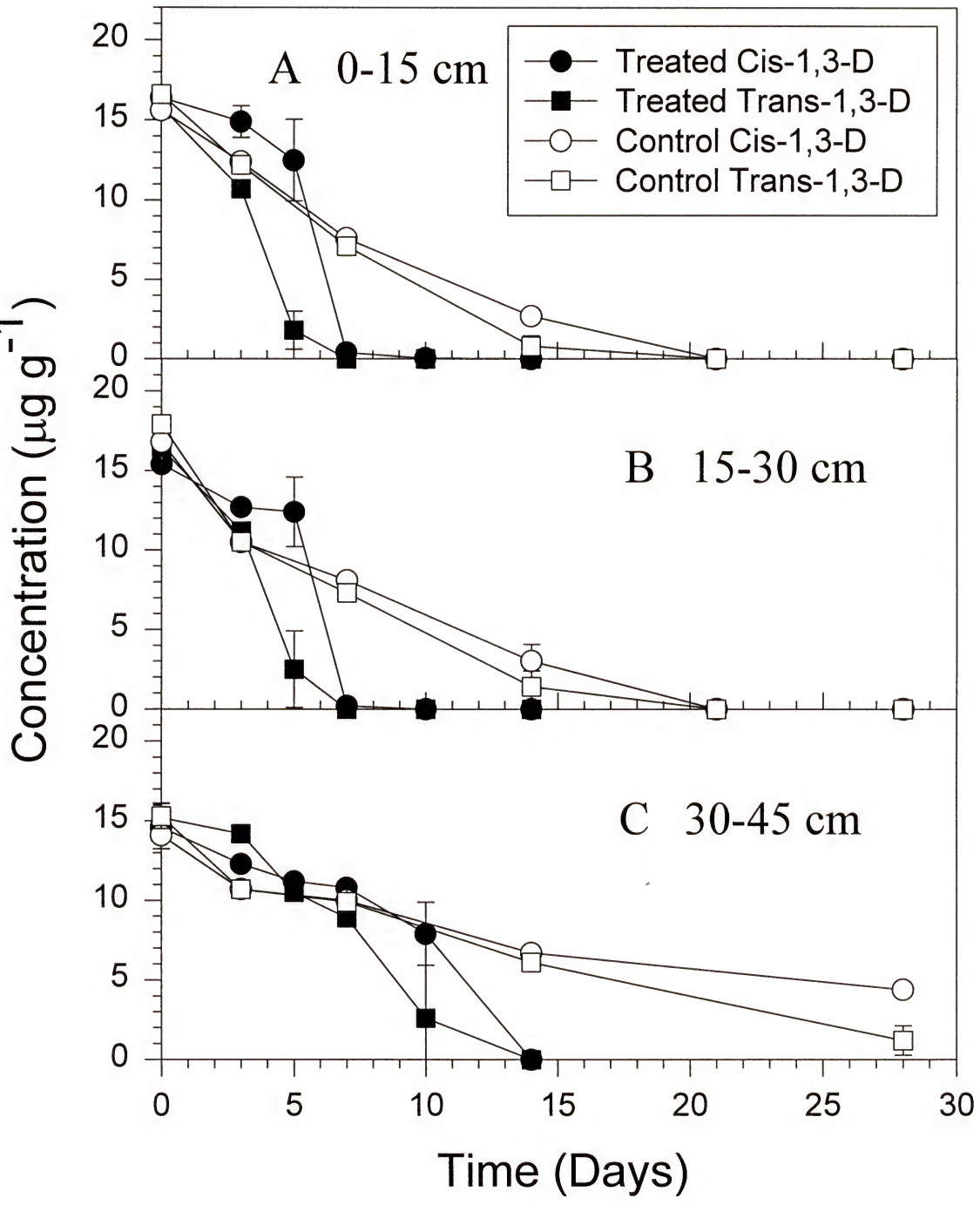


Figure 9. Degradation of cis- and trans-1,3-D in treated and control soils 3 weeks after the 1995 annual application. Error bars represent the 95% confidence intervals.

degraded at a lower rate than cis-1,3-D (Fig. 9C) but, after 3 days, trans-1,3-D was disappearing more rapidly than cis-1,3-D. As a result, the amounts of trans-1,3-D by days 7 and 10 were smaller than for cis-1,3-D. After 10 days, cis-1,3-D was rapidly degraded and, after 14 days, neither isomer was detected.

Cis- and trans-1,3-dichloropropene in the untreated surface and subsurface soils were generally degraded steadily at similar rates during the first 28 days of incubation (Fig. 9). After 21 days, cis- and trans-1,3-D were not detected in the surface and shallow subsurface soils; whereas, at the end of 28 days, small amounts of the two isomers still remained in subsurface soil from the 30-45 cm depth. It is interesting to point out that cis-1,3-D in the treated surface and subsurface soils was initially degraded more slowly than in the corresponding untreated soils and, only after 5 to 7 days, was cis-1,3-D in the treated soils degraded more rapidly than in the untreated soils. Even for trans-1,3-D in the treated subsoils, this isomer was initially degraded more slowly than in the untreated subsoils. After 3 to 5 days, however, trans-1,3-D in the treated soils was degraded more rapidly than in the untreated subsoils.

Six Months after Annual Application

Unlike the treated surface soil collected three weeks after annual application, cis-and trans-1,3-D in the treated

surface soil collected 6 months after annual application were initially during the first 7 days degraded at a similar rate (Fig. 10A). After 7 days, trans-1,3-D was degraded more rapidly than cis-1,3-D. So, after 14 days, both cis- and trans-1,3-D were only barely or not detected. After 21 days, neither isomer was detected in the treated soil. Similar degradation patterns were observed for the treated shallow subsurface soil, in which the two isomers were initially degraded at the same rate and, after 3 days, trans-1,3-D was degraded faster than cis-1,3-D (Fig. 10B). No trans-1,3-D was detected in this soil after 10 days; whereas a small amount of cis-1,3-D was still detected after 14 days. In contrast to the two upper layers of soil, the two isomers in the deeper subsoil (30-45 cm depth) were steadily degraded at a similar rate during the first 21 days (Fig. 10C). After 21 days, neither isomer was detected in this soil. The control soils behaved the same as shown in Fig. 9.

Ten Months after Annual Application

Ten months after annual application, cis- and trans-1,3-D in the treated surface soil and two subsoils were degraded at similar rates during the entire 28 days of incubation (Fig. 11A). To 14 days, cis- and trans-1,3-D in the treated surface soil had been degrading linearly at the same rate. By 14 days, neither isomer remained present in appreciable amounts. Similar degradation patterns were observed for the two treated subsoils (Fig. 11B and C).

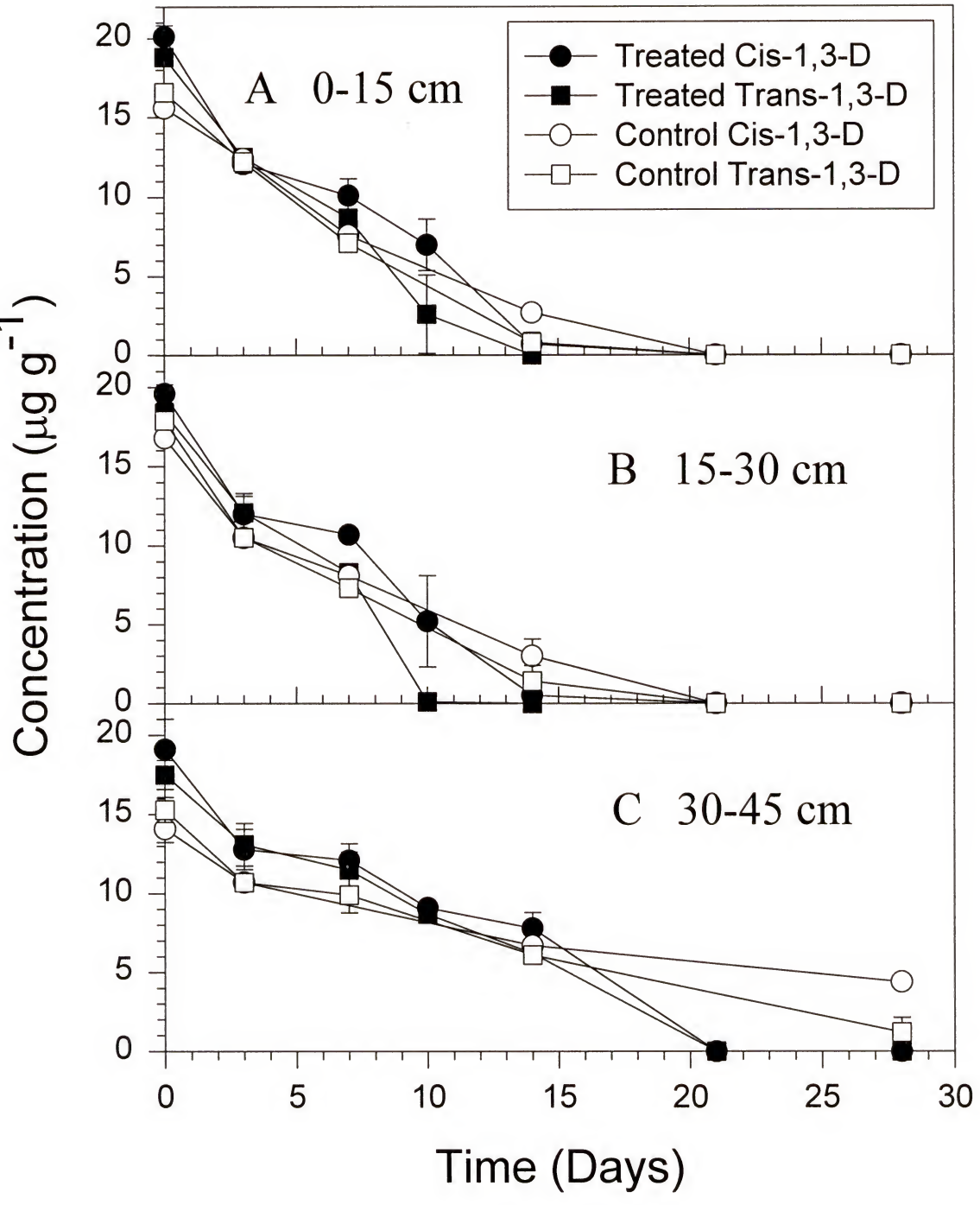


Figure 10. Degradation of cis- and trans-1,3-D in treated and control soils 6 months after the 1995 annual application. Error bars represent the 95% confidence intervals.

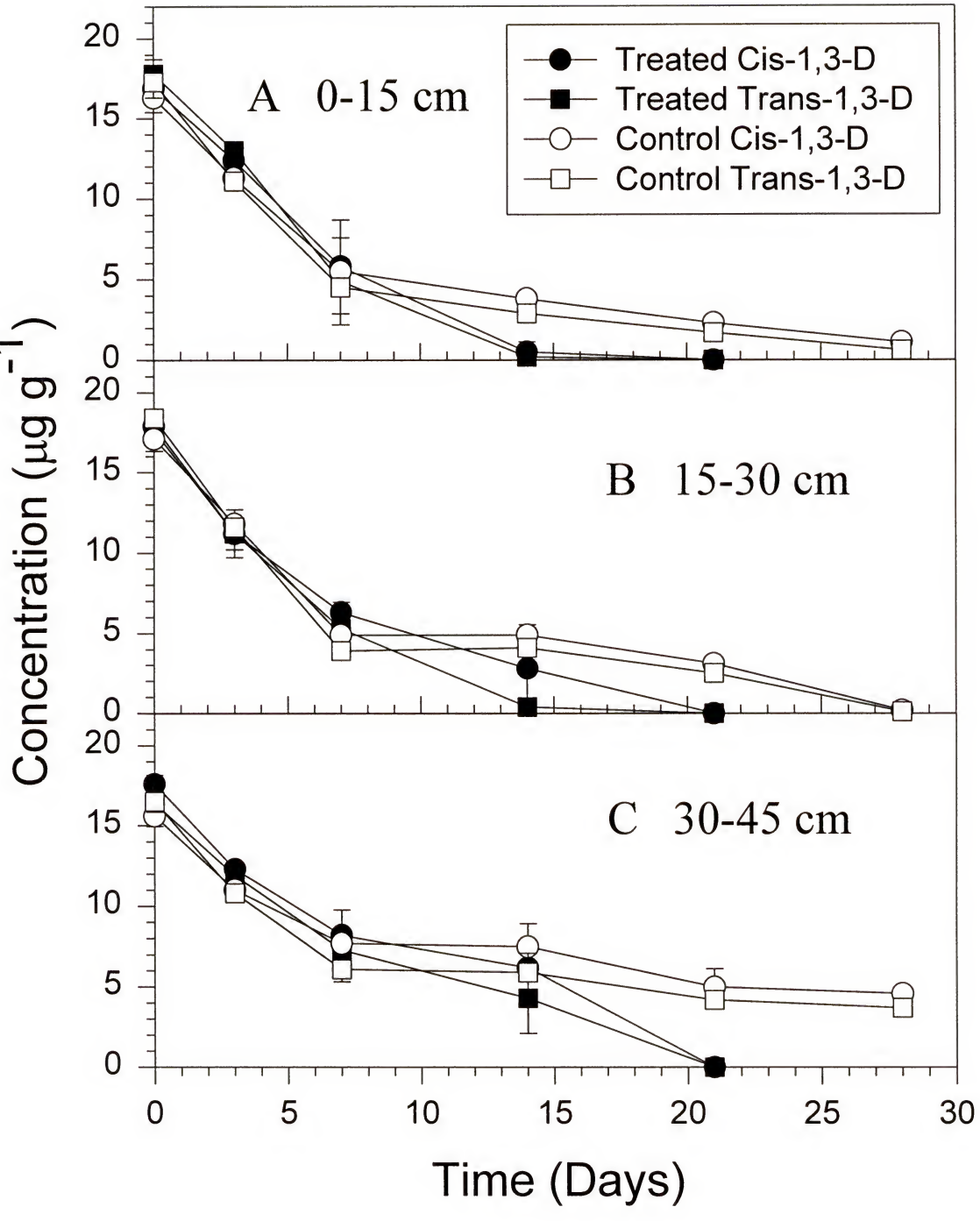


Figure 11. Degradation of cis- and trans-1,3-D in treated and control soils 10 months after the 1995 annual application. Error bars represent the 95% confidence intervals.

Although after 7 days the two isomers in the two subsoils were degrading more slowly than in the surface soil, the isomers were not detected in the subsoils after 21 days.

Similar to the treated soils, cis- and trans-1,3-D in the untreated surface and shallow subsurface soils were linearly degraded at similar rates during the first 7 days (Fig. 11A and B). After 7 days, both isomers degraded more slowly but also linearly or nearly linearly. After 28 days, both isomers were not detected. Both isomers in the untreated deep subsurface soil were more slowly degraded (Fig. 11C). As a result, at the end of 28 days of incubation, 4.6 and 3.7 μg/g of cis- and trans-1,3-D, respectively, still remained in this soil.

Two Years after Annual Application

Two years after the last annual application, cis- and trans-1,3-D in the treated and untreated surface soil and shallow subsoil were degraded during the first 14 days at the same rates (Fig. 12A and B). After 14 days, the two isomers in the two treated soils degraded somewhat faster than in the corresponding untreated soils. As a result, the two isomers were not detected in the two treated soils after 21 days; whereas 3 to 5 μg/g of the isomers still remained in the untreated soils. Both cis- and trans-1,3-D in the treated and untreated deeper subsoils were degraded at similar rates (Fig. 12C). During the first 14 days, the two

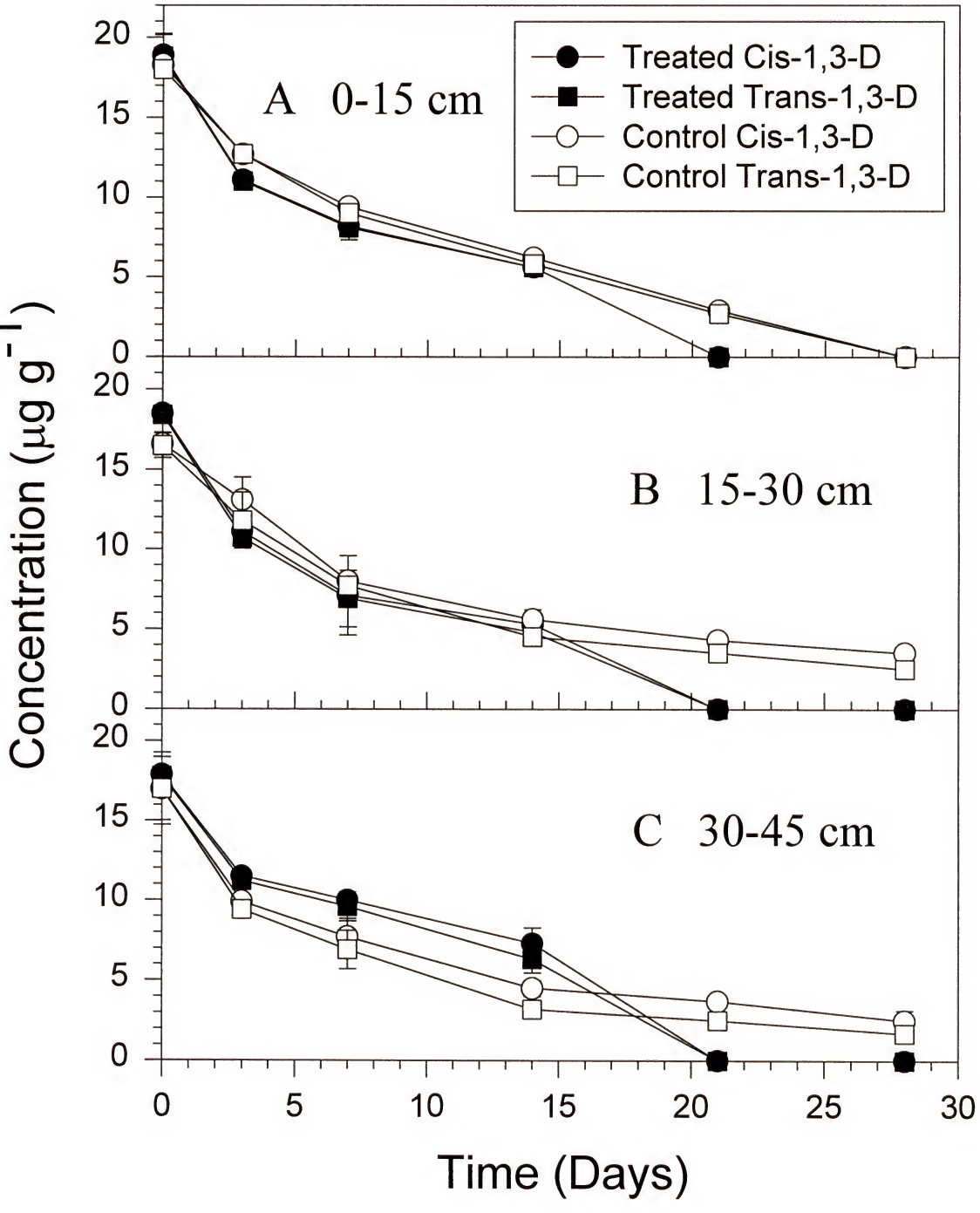


Figure 12. Degradation of cis- and trans-1,3-D in treated and control soils 2 years after the 1995 annual application. Error bars represent the 95% confidence intervals.

isomers in the treated soil degraded more slowly than in the untreated soil. After 14 days, the situation changed, with the two isomers in the treated soil degrading much faster than in the untreated soil. As a result, no cis- or trans-1,3-D was detected in the treated soil after 21 days, whereas small amounts of the isomers still remained in the untreated soil after 28 days.

It appears that, two years after the last application, enhanced degradation of cis- and trans-1,3-D in the treated soils decreased to near the same level as in the untreated soils. Thus, duration of enhanced degradation of cis- and trans-1,3-D in this soil appears to be slightly more than 2 years.

Degradation of Cis- and Trans-1,3-D in Soil Treated with 1,3-D in 1996

Three Weeks after Annual Application

Trans-1,3-dichloropropene in the treated surface soil and shallow subsurface soil collected 3 weeks after annual application was degraded more rapidly during the first 3 days of incubation than cis-1,3-D (Fig. 13A and B). After 3 days, cis-1,3-D was more rapidly degraded. As a result, after 7 days, both isomers had completely degraded and were not detected thereafter in the two soils. Cis- and trans-1,3-D in the subsurface soil of 30-45 cm depth degraded at a similar rate during the first 3 days of incubation (Fig. 13C) but, after 3 days, trans-1,3-D degraded more rapidly than cis-1,3-D. After 7 days, cis-1,3-D more rapidly

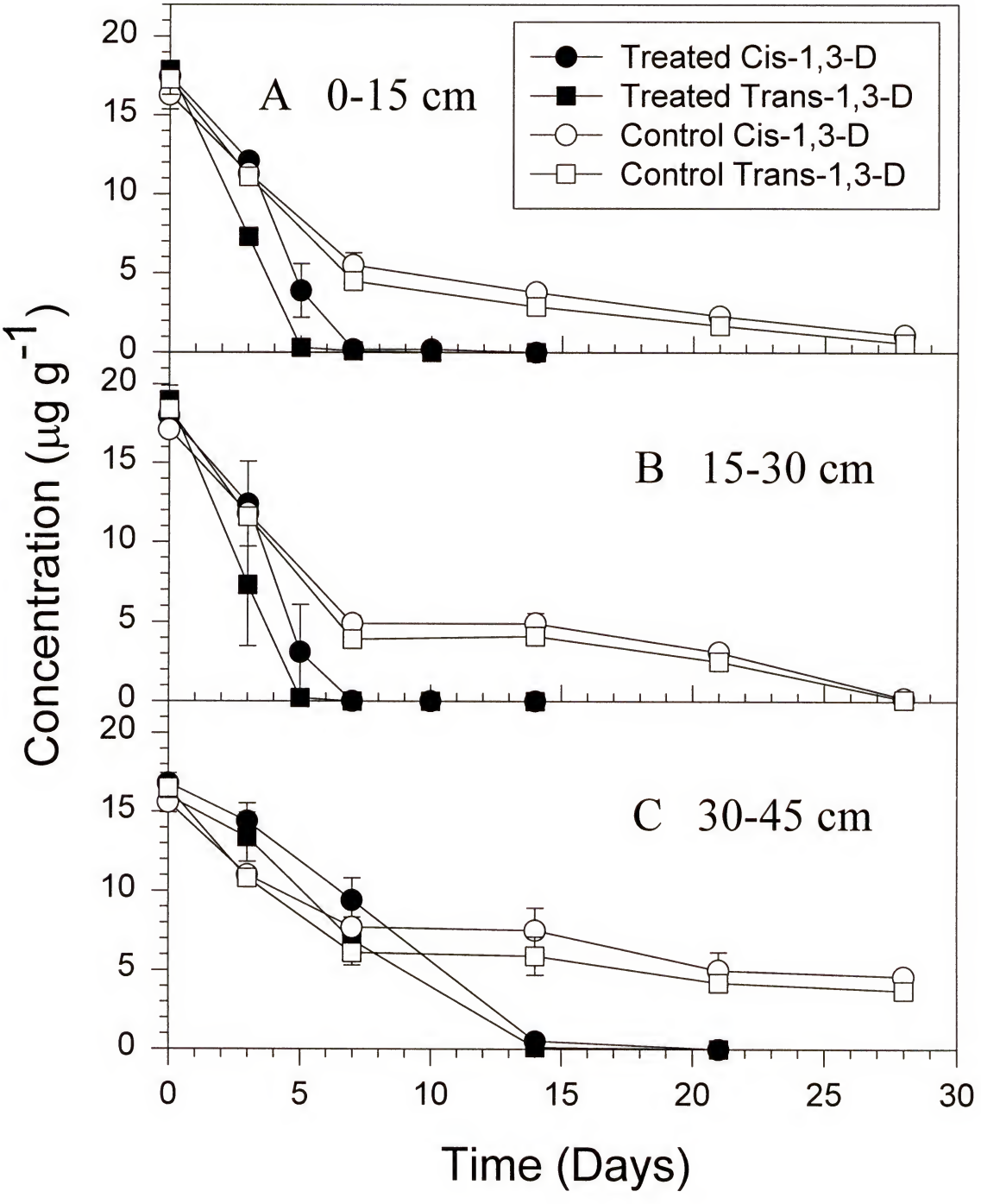


Figure 13. Degradation of cis- and trans-1,3-D in treated and control soils 3 weeks after the 1996 annual application. Error bars represent the 95% confidence intervals.

degraded. As a result, after 14 days, both isomers nearly or completely degraded and, after 21 days, the isomers were no longer detected.

With the exception of cis-1,3-D during the first 3 days of incubation, cis- and trans-1,3-D in the treated surface soil and shallow subsoil were degraded faster than in the corresponding untreated soils (Fig. 13A and B). In the deeper treated subsoil, however, the two isomers degraded similarly or more slowly during the first 7 days than for the corresponding untreated soil (Fig. 13C). After 7 days the situation had changed, and they now degraded faster than in the untreated soil. Furthermore, after one additional annual application of 1,3-D, enhanced degradation of cis- and trans-1,3-D in the treated soil was more profound than in the same soil that had been treated with 1,3-D one year earlier.

Five Months after Annual Application

Trans-1,3-dichloropropene in the treated surface soil and shallow subsoil was degraded faster than cis-1,3-D during the first 7 days of incubation (Fig. 14A and B). However, the degree of difference was smaller than for the samples collected three weeks after the annual application. Cis- and trans-1,3-D in the treated and untreated surface and shallow subsurface soils degraded generally at similar rates during the first 7 days of incubation (Fig. 14A and B). However, after 7 days the two isomers in the treated

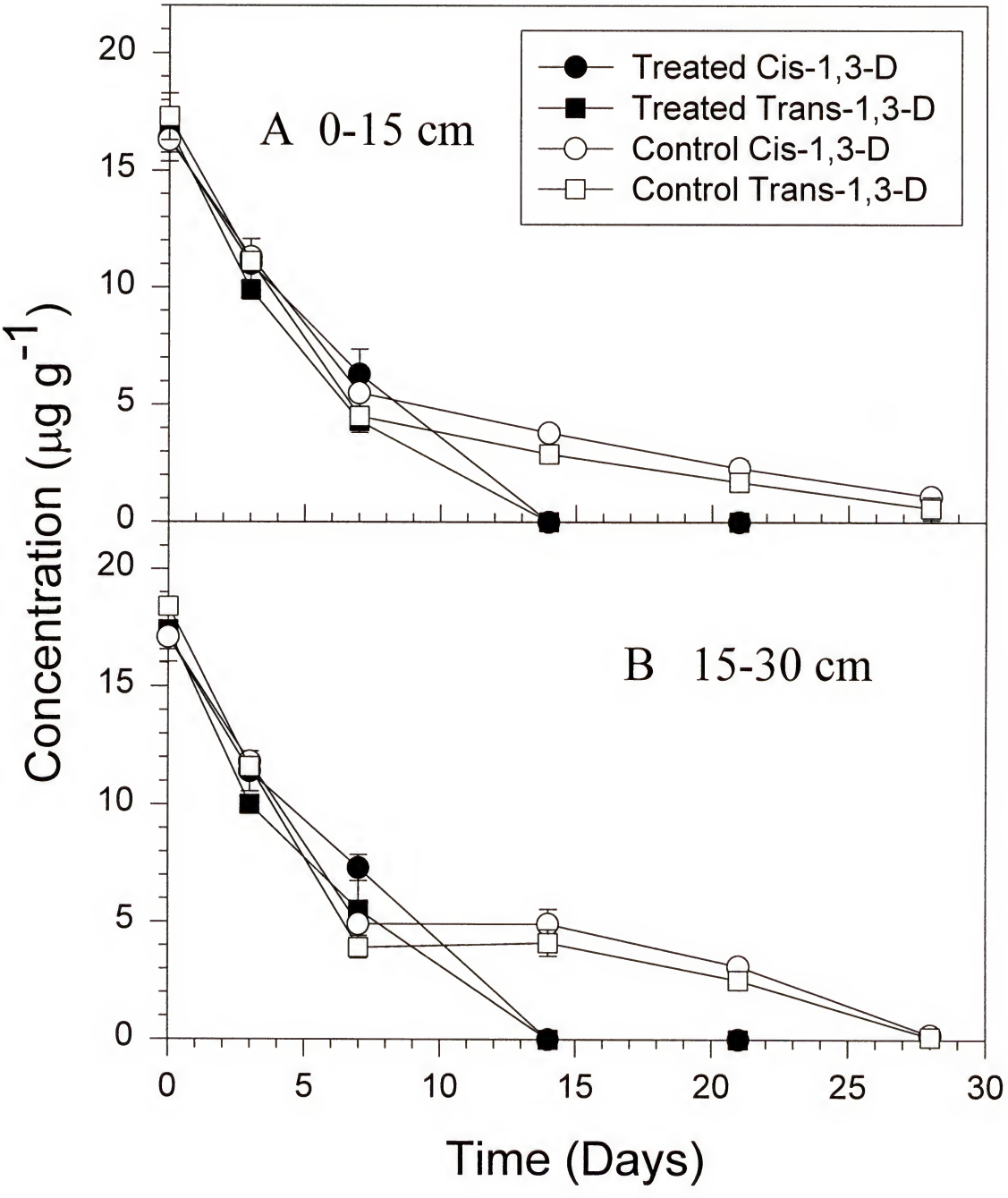


Figure 14. Degradation of cis- and trans-1,3-D in treated and control soils 5 months after the 1996 annual application. Error bars represent the 95% confidence intervals.

soils degraded faster than in the corresponding untreated soils, resulting in complete disappearance of cis- and trans-1,3-D from the treated soils after 14 days. Degradation of the two isomers was not determined for the deeper treated soil.

Ten Months after Annual Application

Both cis- and trans-1,3-dichloropropene in the treated surface soil and the two subsoils were generally degraded at similar rates throughout the entire 28 days of incubation (Fig. 15). No cis- or trans-1,3-D was detected in the treated surface, shallow subsurface and deeper subsurface soils after 14, 14, and 28 days, respectively. Cis- and trans-1,3-D in the treated surface soil and the two subsoils initially degraded at the same rate as for the corresponding control soils (Fig. 15). After 3, 7, and 21 days, however, the two isomers in treated surface, shallow subsurface and deeper subsurface soils, respectively, degraded faster than in the corresponding untreated soils. Similar to the 1995 treated soils, enhanced degradation of cis- and trans-1,3-D in the treated soils progressively declined with time.

Degradation of Cis- and Trans-1,3-D in Soil Treated with 1,3-D in 1997

Three Weeks after Annual Application

Both cis- and trans-1,3-D were degraded faster in 1997-treated surface soil and the two subsoils than in the corresponding 1996-treated soils. In fact, enhanced degradation of the two isomers in the treated soil

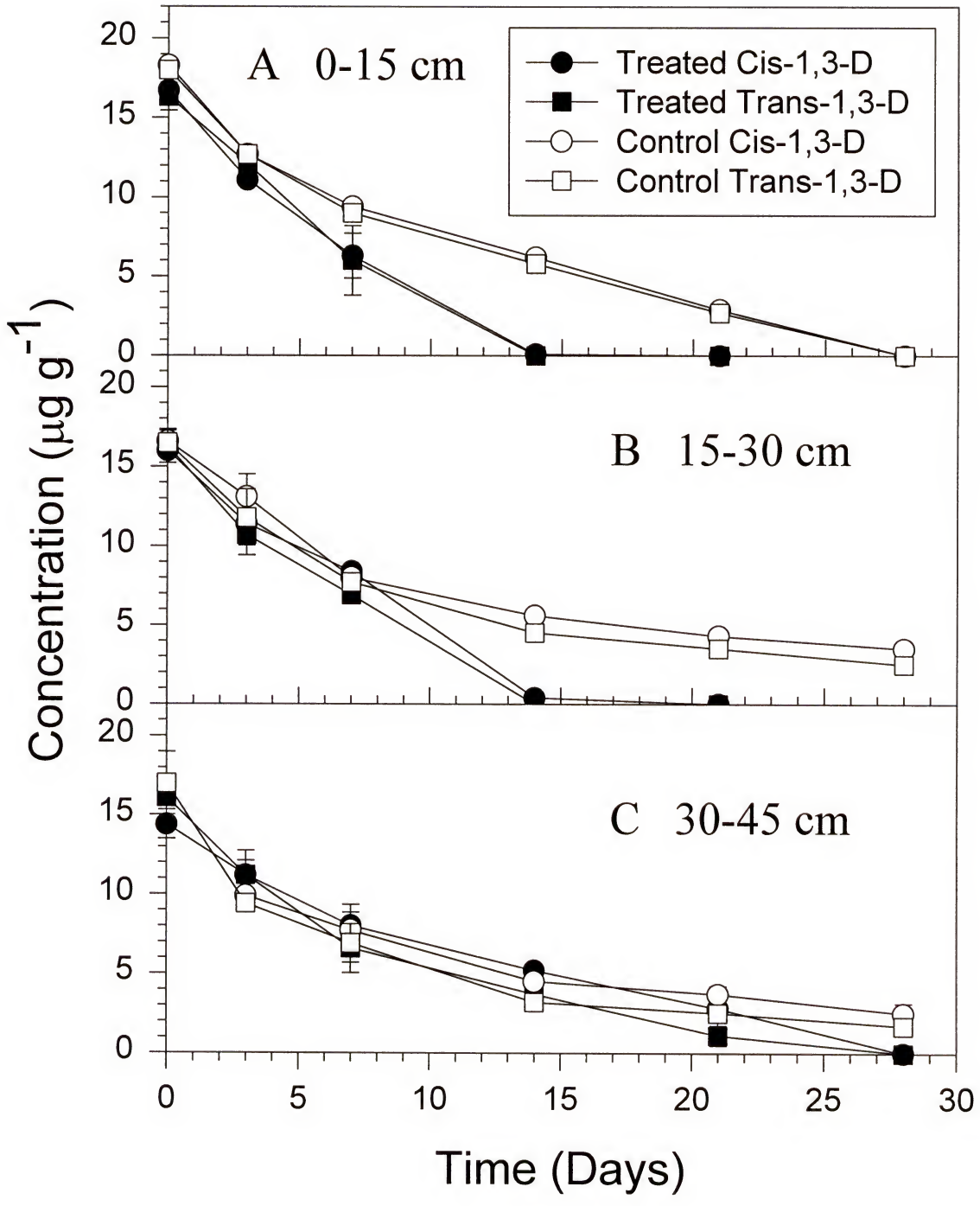


Figure 15. Degradation of cis- and trans-1,3-D in treated and control soils 10 months after the 1996 annual application. Error bars represent the 95% confidence intervals.

progressively increased with number of annual applications. Cis- and trans-1,3-D in the treated surface soil and shallow subsoil were degraded faster than in the corresponding control soil and, after 7 days, they were no longer detected (Fig. 16A and B). For the deeper treated subsoil, cis- and trans-1,3-D were initially degraded similarly as in the control subsoil (Fig. 16C) but, after 7 days, they were degraded faster than in the untreated subsoil. No cis- and trans-1,3-D were detected in the treated soil after 14 days.

Degradation of Cis- and Trans-1,3-D in Retreated Soils

It appeared that, two years after the last 1,3-D application, 1,3-D degrading microorganisms were still present in the treated soil. Three weeks after the retreatment with 1,3-D, both cis- and trans-1,3-D in the retreated surface soil and the two subsoils were degrading faster than in the corresponding soils collected two years after last application or in the control soils (Fig. 17). Enhanced degradation of cis- and trans-1,3-D in the retreated soils was not as great as in the treated soils collected 3 weeks after the 1997 application, however.

Degradation of Cis- and Trans-1,3-D in Autoclaved Soils

To determine whether cis- and trans-1,3-D in untreated soils were mainly degraded chemically, treated surface and shallow subsoil collected 5 months after the 1996 annual application were sterilized by autoclaving. Unlike in nonsterile treated soils (see Fig. 15), cis- and trans-1,3-D

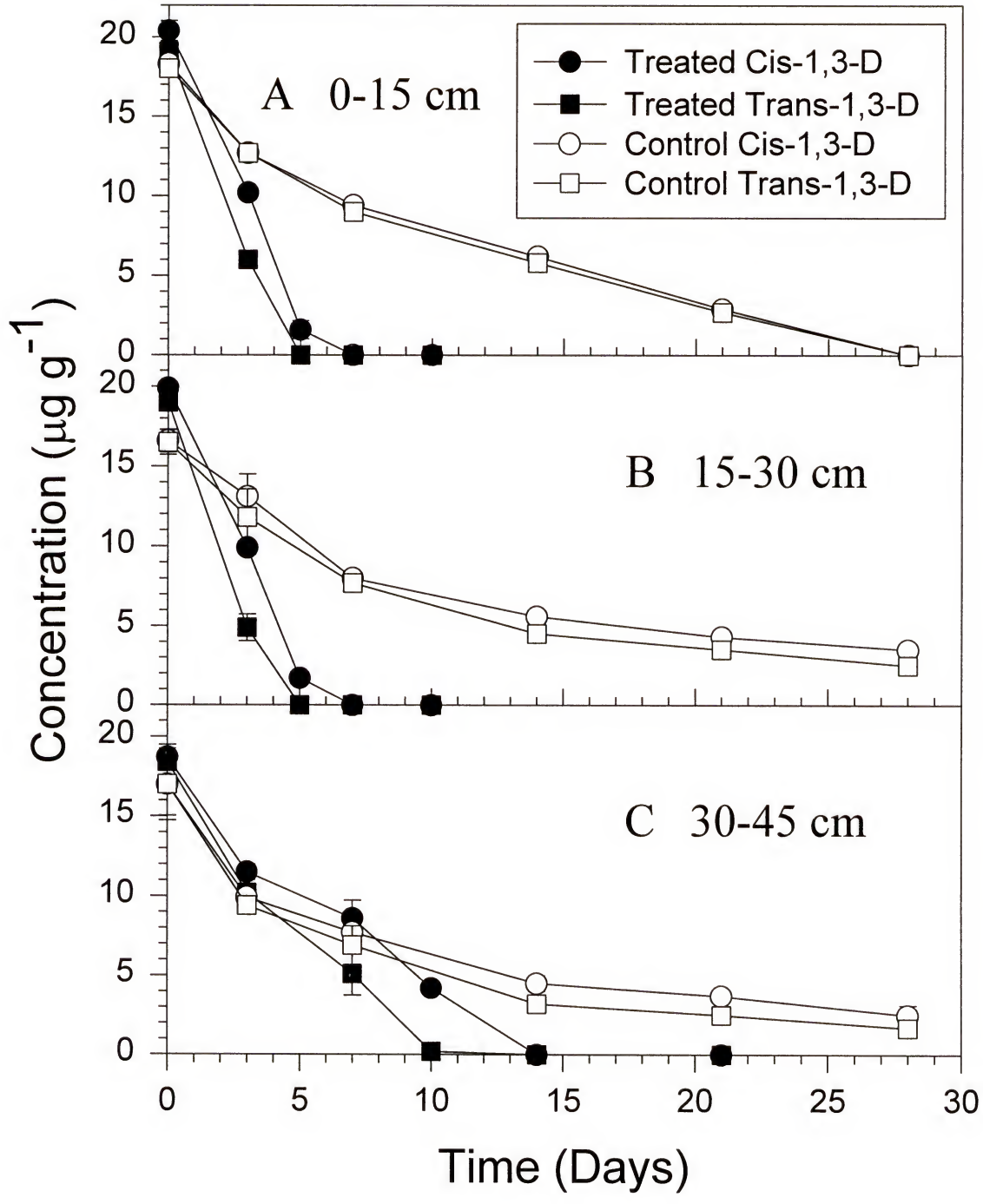


Figure 16. Degradation of cis- and trans-1,3-D in treated and control soils 3 weeks after the 1997 annual application. Error bars represent the 95% confidence intervals.

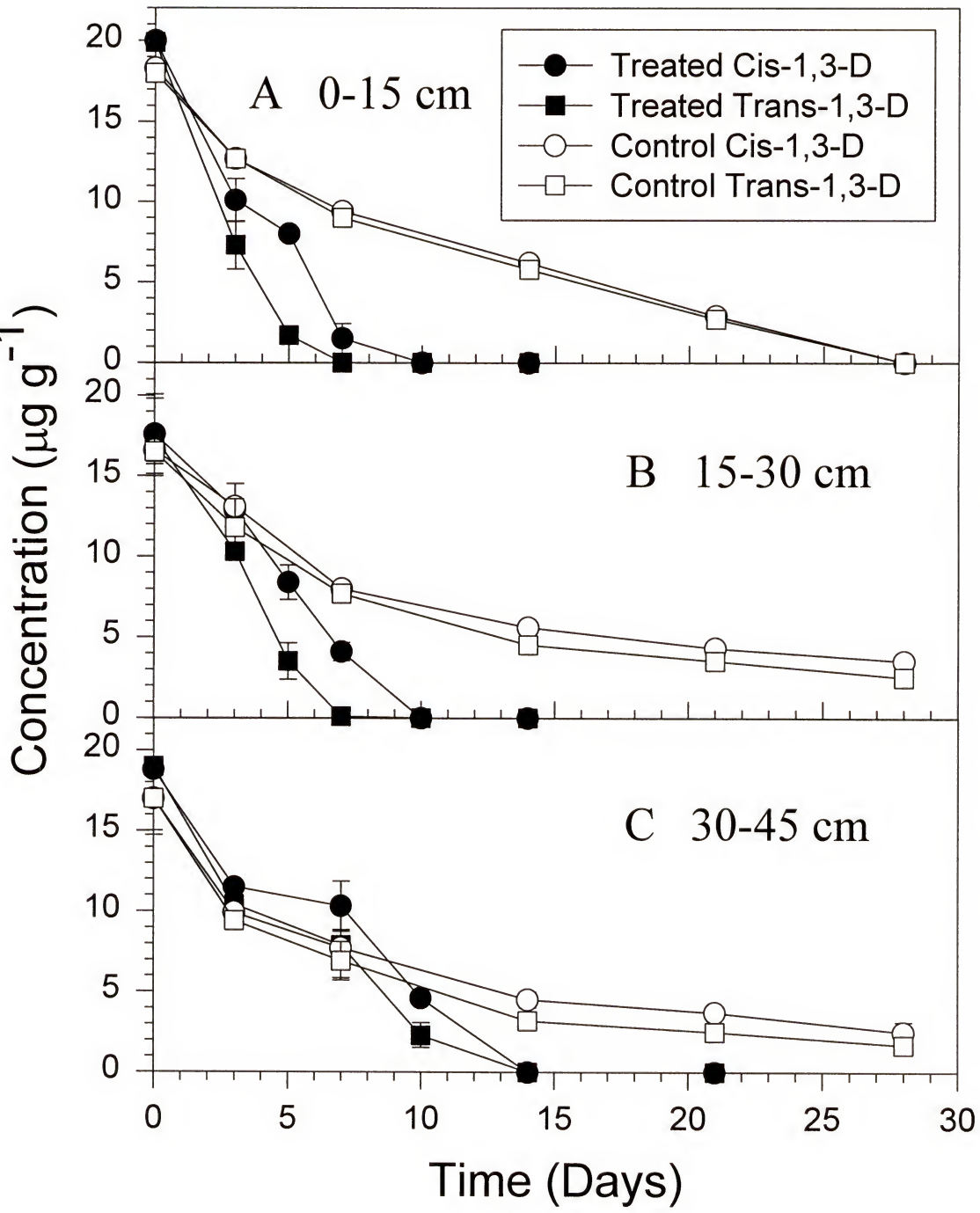


Figure 17. Degradation of cis- and trans-1,3-D in retreated and control soils 3 weeks after reapplication to soils that had not been treated with 1,3-D for 2 years. Error bars represent the 95% confidence intervals.

in the autoclaved treated surface soil and subsoil did not completely degrade in 14 days (Fig. 18). In fact, the two isomers in the autoclaved soils degraded following similar patterns as for the control soils. Therefore, cis- and trans-1,3-D in untreated soils appears to be principally degraded chemically, and contributions from microbial degradation were likely negligible.

Half-life Values of Cis- and Trans-1,3-D in Treated and Untreated soils

Half-life values for trans-1,3-D in treated and control surface soil (0-15 cm of depth) and shallow subsoil (15-30 cm of depth) and deep subsoil (30-45 cm of depth) collected 3 weeks and 6 months after the 1995 annual application of 1,3-D were significantly smaller than those for cis-1,3-D, with the exception that the half-life values for the two isomers in the deep subsoil collected 6 months after the annual application were not significantly different (Table 5). The half-life values for the two isomers in the treated surface soil were not significantly different from those for the treated shallow subsoil. In contrast to the upper two layers of soil, half-life values for the two isomers in the deeper subsoil were significantly larger than for the top two soils. The half-life values for the two isomers in the 3 layers of the treated soil were significantly smaller than for the corresponding layers of the control soil collected 3 weeks and 6 months after the annual application, respectively.

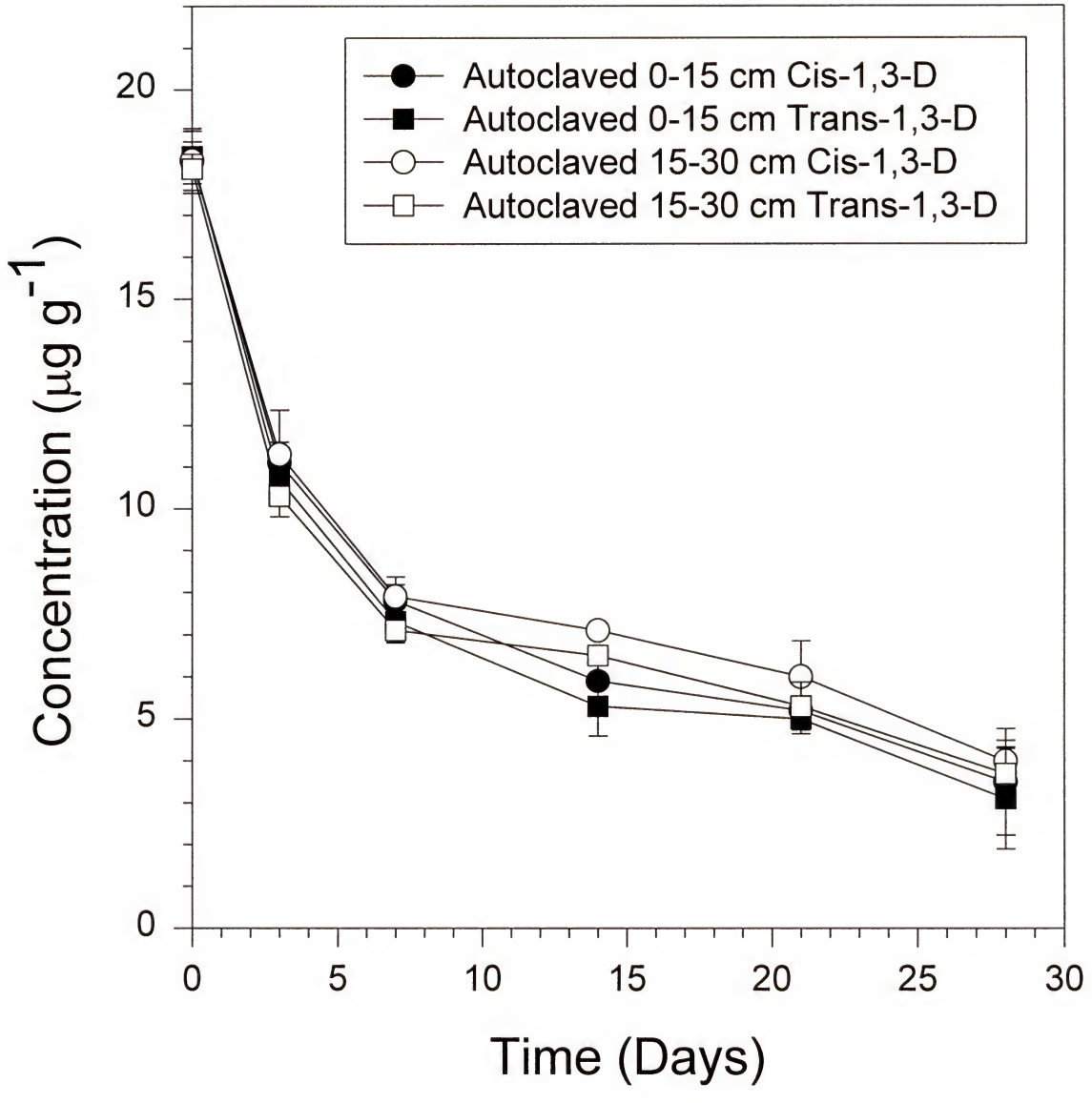


Figure 18. Degradation of cis- and trans-1,3-D in autoclaved treated surface and subsurface soils. Error bars represent the 95% confidence intervals.

Table 5. Pseudo-first-order rate coefficients (K_1) and half-life ($t_{1/2}$) values of cis- and trans-1,3-D in surface (0-15 cm), shallow and deep subsurface (15-30 and 30-45 cm) soil samples collected from treated and untreated (control) plots in 1995 at the Green Acres site, Gainesville, Florida. These samples were treated with an equal ratio of cis- and trans-1,3-D.

Field application in 1995

Time after application (months)	Soil depth (cm)	K_1 (1/days)		$t_{1/2}$ (days)		r^2	
		Cis[a]	Trans[b]	Cis[a]	Trans[b]	Cis[a]	Trans[b]
1995		Control					
0.7	0-15	0.135±0.005	0.161±0.025	5.16±0.01	4.33±0.69	0.994	0.981
	15-30	0.133±0.009	0.149±0.019	5.24±0.37	4.69±0.60	0.989	0.955
	30-45	0.084±0.001	0.105±0.000	8.33±0.11	6.63±0.00	0.884	0.931
		Treated					
0.7	0-15	0.345±0.014	0.475±0.140	2.01±0.08	1.50±0.44	0.606	0.897
	15-30	0.335±0.003	0.597±0.149	2.07±0.02	1.18±0.29	0.597	0.760
	30-45	0.171±0.010	0.207±0.008	4.06±0.23	3.35±0.13	0.724	0.905
6	0-15	0.215±0.000	0.348±0.013	3.23±0.01	2.00±0.07	0.821	0.943
	15-30	0.246±0.042	0.464±0.003	2.85±0.48	1.50±0.01	0.810	0.702
	30-45	0.126±0.001	0.127±0.001	5.53±0.03	5.49±0.04	0.831	0.874
10	0-15	0.260±0.040	0.356±0.054	2.69±0.43	1.96±0.29	0.954	0.946
	15-30	0.131±0.014	0.284±0.022	5.28±0.57	2.45±0.19	0.998	0.962
	30-45	0.125±0.003	0.126±0.008	5.58±0.15	5.43±0.35	0.899	0.963
24	0-15	0.126±0.002	0.126±0.003	5.52±0.09	5.54±0.15	0.918	0.917
	15-30	0.127±0.007	0.126±0.008	5.49±0.26	5.52±0.32	0.926	0.941
	30-45	0.122±0.003	0.123±0.001	5.70±0.12	5.63±0.06	0.852	0.885

Cis[a]= Cis-1,3-D, Trans[b]= Trans-1,3-D.
The ± numbers in Tables 5, 6, 7, and 8 are the 95% confidence intervals for the mean.

Half-life values of cis- and trans-1,3-D for the 3 layers of the treated soil collected 10 months after the 1995 annual application were significantly smaller than for the corresponding control soils (Table 5). The half-life value for cis-1,3-D in the treated surface soil was significantly smaller than those for the treated shallow subsoil and deep subsoil. However, the half-life value for cis-1,3-D in the treated shallow subsoil was not significantly different from that for the treated deep subsoil. The half-life value for trans-1,3-D in treated surface soil also was not significantly different from that for the shallow subsoil. The half-life values of trans-1,3-D for the upper two layers of the treated soil were significantly smaller than for the treated deep subsoil (Table 5). The half-life values for trans-1,3-D also were significantly smaller than those for cis-1,3-D in the treated shallow subsoil and 3 depths of the control soil. However, the half-life value for trans-1,3-D in the treated surface soil was not significantly different from that for the treated deep subsoil.

The half-life value for cis-1,3-D in the control surface soil was not significantly different from that for the control shallow subsoil. The half-life values for cis-1,3-D in the upper two layers of the control soil were significanlty smaller than those for the deeper subsoil, whereas the half-life value for trans-1,3-D in the control

surface soil was significantly smaller than for the control shallow subsoil, and the half-life value for the isomer in the shallow subsoil was significantly smaller than for the deep subsoil.

Half-life values for cis- and trans-1,3-D in the treated soils collected 24 months after the 1995 annual application were significantly smaller than for the corresponding control soils, with the exception that the half-life value for cis-1,3-D in the treated surface soil was not significantly different from the value for the corresponding control surface soil (Table 5). The half-life values for trans-1,3-D in the 3 layers of the treated soil were not significantly different from those for cis-1,3-D.

The half-life values for trans- and cis-1,3-D in the control surface soil were not significantly different from each other. However, the half-life values for trans-1,3-D in the two lower layers of the control soil were significantly smaller than the corresponding half-life values for cis-1,3-D. The half-life values for cis-1,3-D in the two sublayers of the control were not significantly different from each other. However, the half-life value for cis-1,3-D in the control surface soil was significantly smaller than values for the two lower layers of the control soil. The half-life value for trans-1,3-D in the control surface soil was also significantly smaller than that for the control deep subsoil, and the half-life value for the isomer in the

control deep subsoil was significantly smaller than that for the control shallow subsoil.

Half-life values for cis- and trans-1,3-D in the 3 layers of the treated soil collected 3 weeks after the 1996 annual application and for the 2 upper layers of the treated soil 5 months after the 1996 annual application were significantly smaller than for the corresponding control soils (Table 6). The half-life values of trans-1,3-D in the two lower layers of the treated soil collected 3 weeks after the 1996 annual application were significantly smaller than those for cis-1,3-D, whereas the half-life value of trans-1,3-D in the treated surface soil was not significantly different from that for cis-1,3-D. In addition, the half-life values of trans-1,3-D in the treated surface soil and shallow subsoil collected 5 months after the 1996 annual application were not significantly different from those for cis-1,3-D. The half-life value of cis-1,3-D in the treated surface soil collected 3 weeks after the 1996 annual application was not significantly different from that for the treated shallow subsoil, and the half-life value of cis-1,3-D in the treated shallow subsoil was not significantly different from the treated deep subsoil. However, the half-life value of cis-1,3-D in the treated surface soil was significantly smaller than in the treated deep subsoil. The half-life values for trans-1,3-D in the treated surface soil and shallow subsoil collected 3 weeks after the 1996 annual

Table 6. Pseudo-first-order rate coefficients (K_1) and half-life ($t_{1/2}$) values of cis- and trans-1,3-D in surface (0-15 cm), shallow and deep subsurface (15-30 and 30-45 cm) soil samples collected from treated and untreated (control) plots in 1996 at the Green Acres site, Gainesville, Florida. These samples were treated with an equal ratio of cis- and trans-1,3-D.

Field application in 1996

Time after application (months)	Soil depth (cm)	K_1 (1/days)		$t_{1/2}$ (days)		r^2	
		Cis[a]	Trans[b]	Cis[a]	Trans[b]	Cis[a]	Trans[b]
1996		Control					
0.7	0-15	0.092±0.013	0.112±0.001	7.61±1.07	6.22±0.05	0.975	0.969
	15-30	0.077±0.003	0.091±0.001	9.10±0.35	7.68±0.07	0.842	0.833
	30-45	0.046±0.007	0.059±0.005	15.17±2.25	11.83±1.00	0.896	0.848
		Treated					
0.7	0-15	0.603±0.041	0.797±0.035	1.15±0.08	0.87±0.04	0.789	0.827
	15-30	0.414±0.007	0.861±0.010	1.68±0.02	0.81±0.00	0.906	0.820
	30-45	0.258±0.027	0.372±0.006	2.70±0.28	1.87±0.03	0.884	0.884
5	0-15	0.201±0.001	0.204±0.005	3.45±0.02	3.41±0.08	0.971	0.982
	15-30	0.203±0.000	0.204±0.001	3.42±0.00	3.41±0.03	0.955	0.999
10	0-15	0.202±0.002	0.204±0.002	3.43±0.03	3.40±0.03	0.988	0.992
	15-30	0.198±0.006	0.199±0.008	3.50±0.10	3.48±0.14	0.938	0.958
	30-45	0.091±0.004	0.108±0.013	7.64±0.34	6.46±0.73	0.976	0.965

Cis[a]= Cis-1,3-D, Trans[b]= Trans-1,3-D.
The ± numbers in Tables 5, 6, 7, and 8 are the 95% confidence intervals for the mean.

application were not significantly different from each other. However, the half-life values for trans-1,3-D in the two upper layers of the treated soil were significantly smaller than the value in the treated deep subsoil. In addition, the half-life values for trans-1,3-D in the treated surface soil and subsoil collected 5 months after the 1996 annual application were not significantly different from the values for cis-1,3-D.

The half-life values for cis- and trans-1,3-D in the 3 depths of the treated soil collected 10 months after the 1996 annual application were significantly smaller than the values for the corresponding control soils (Table 6). The half-life values for the two isomers in the treated surface soil were not significantly different from the values for the treated shallow subsoil, but the half-life values of the two isomers in the treated surface soil and shallow subsoil were signicantly different from the values for the treated deep subsoil. The half-life values for trans-1,3-D in the treated surface and shallow subsoils were not significantly different from those for cis-1,3-D, with the exception that the value for trans-1,3-D was significantly smaller than that for cis-1,3-D in the treated deep subsoil.

The half-life values for cis- and trans-1,3-D in the 3 layers of the treated soil collected 3 weeks after the 1997 annual application were significantly smaller than the values for the corresponding control soils (Table 7). The

Table 7. Pseudo-first-order rate coefficients (K_1) and half-life ($t_{1/2}$) values of cis- and trans-1,3-D in surface (0-15 cm), shallow and deep subsurface (15-30 and 30-45 cm) soil samples collected from treated and untreated (control) plots in 1997 at the Green Acres site, Gainesville, Florida. These samples were treated with an equal ratio of cis- and trans-1,3-D.

Field application in 1997

Time after application (months)	Soil depth (cm)	K_1 (1/days)		$t_{1/2}$ (days)		r^2	
		Cis[a]	Trans[b]	Cis[a]	Trans[b]	Cis[a]	Trans[b]
1997		Control					
0.7	0-15	0.098±0.001	0.099±0.001	7.11±0.09	7.06±0.09	0.971	0.982
	15-30	0.056±0.001	0.066±0.001	12.52±0.15	10.54±0.24	0.922	0.957
	30-45	0.064±0.016	0.082±0.011	11.02±2.91	8.55±1.06	0.989	0.972
		Treated					
0.7	0-15	0.467±0.016	0.866±0.000	1.49±0.05	0.80±0.00	0.867	0.854
	15-30	0.461±0.003	0.997±0.014	1.51±0.00	0.70±0.00	0.876	0.857
	30-45	0.332±0.000	0.441±0.041	2.09±0.00	1.58±0.15	0.765	0.782

Cis[a]= Cis-1,3-D, Trans[b]= Trans-1,3-D.
The ± numbers in Tables 5, 6, 7, and 8 are the 95% confidence intervals for the mean.

half-life values for trans-1,3-D in the treated surface soil and deeper subsoil were not significantly different from the values for cis-1,3-D, with the exception that the half-life value for trans-1,3-D was significantly smaller than the value for cis-1,3-D in the treated shallow subsoil.

The half-life values for cis- and trans-1,3-D in the 3 layers of retreated soil collected 3 weeks after the 1997 reapplication to the soil that had not been treated with 1,3-D for 2 years were significantly smaller than the values for the corresponding control soils (Table 8). The half-life values for cis-1,3-D in the 3 layers of the treated soil were not significantly different from each other. However, the half-life values for trans-1,3-D in the retreated surface soil and shallow subsoil were significantly smaller than the value for the retreated deeper subsoil, though the half-life value for trans-1,3-D in the retreated surface soil was not significantly different from the value for the retreated shallow subsoil. The half-life values for cis- and trans-1,3-D in the retreated surface soil and deeper subsoil were not significantly different from each other, with the exception that the half-life value for trans-1,3-D was significantly smaller than the value for cis-1,3-D in the retreated shallow subsoil.

Table 8. Pseudo-first-order rate coefficients (K_1) and half-life ($t_{1/2}$) values of cis- and trans-1,3-D in autoclaved treated surface (0-15 cm), shallow subsurface (15-30 cm) soil samples in 1996 and retreated surface, shallow subsurface and deep subsurface soil samples in 1997 at the Green Acres site, Gainesville, Florida. These samples were treated with an equal ratio of cis- and trans-1,3-D.

Time after application (months)	Soil depth (cm)	K_1 (1/days)		$t_{1/2}$ (days)		r^2	
		Cis[a]	Trans[b]	Cis[a]	Trans[b]	Cis[a]	Trans[b]
1996		Autoclaved Soil					
5	0-15	0.053±0.013	0.057±0.014	13.45±3.45	12.43±2.98	0.907	0.893
	15-30	0.046±0.001	0.048±0.003	15.10±0.34	14.56±0.96	0.887	0.865
1997		Retreated Soil					
0.7	0-15	0.343±0.005	0.481±0.087	2.03±0.03	1.46±0.26	0.803	0.952
	15-30	0.286±0.008	0.690±0.007	2.43±0.07	1.00±0.00	0.914	0.906
	30-45	0.195±0.000	0.198±0.002	3.56±0.00	3.50±0.04	0.900	0.948

Cis[a]= Cis-1,3-D, Trans[b]= Trans-1,3-D.
The ± numbers in Tables 5, 6, 7, and 8 are the 95% confidence intervals for the mean.

The half-life values for trans-1,3-D in the autoclaved treated surface soil and subsoil were not significantly different from the values for cis-1,3-D (Table 8).

Cis- and trans-1,3-D measurements were analyzed using PROC GLM (SAS Institute, Inc., 1989), according to the two-way model with treatments, depths, and their interaction considered to be fixed effects. Measurements at various depths were regarded to be independent. So, analysis of variance for repeated measurements was not used. Differences among treatments and depths were further analyzed by using Fisher's Least Significant Difference Multiple Comparison procedure. Values of p under 0.05 were considered to be significant. Three responses of interest were analyzed: cis-1,3-D, trans-1,3-D, and the difference between cis- and trans-1,3-D. Data were analyzed separately by year, and relevant differences from year to year were also examined.

Statistically, two-way factorial design was used, with treatment as one factor and depth as another factor.

Screening for Biodegradable Organic Compounds that Stimulate Degradation of Cis- and Trans-1,3-D by the Mixed Culture

As mentioned in the Materials and Methods section, cis- and trans-1,3-D were also degraded in basal mineral-trace mineral medium (BMTMM) containing a small amount of nonsterile enhanced soil, but not in the same medium containing autoclaved enhanced soil (Table 9). Even though 1,3-D was degraded in BMTMM supplemented with a small amount of nonsterile enhanced soil, no visible turbidity was

Table 9. Degradation of cis- and trans-1,3-D in basal mineral-trace mineral medium supplemented with nonsterile enhanced soil (10 mg/mL) or autoclaved enhanced soil (10 mg/mL).

Medium	Degradation
Basal mineral-trace mineral medium (BMTMM) + nonsterile enhanced soil	+
BMTMM + autoclaved enhanced soil	-
BMTMM + autoclaved enhanced soil + nonsterile enhanced soil[a]	+

[a] Nonsterile enhanced soil (10 mg/mL) was added 7 days after autoclaved soil was added.

developed in the medium. Development of turbidity is a sign of bacterial growth. Furthermore, inoculation of nonsterile enhanced soil to BMTMM amended with autoclaved enhanced soil resulted in degradation of 1,3-D. It was obvious that the enhanced soil not only contained 1,3-D degrading bacteria but also provided an essential second carbon substrate that was capable of stimulating 1,3-D degradation. Without the second carbon substrate, the 1,3-D degrading bacteria could not degrade the chemical. As also mentioned in the Materials and Methods section, a mixed bacterial culture was isolated, and the mixed culture had the capacity to degrade 1,3-D when grown in BMTMM supplemented with a small amount of soil leachate sterilized by filtration (Table 10). Even though the mixed culture grew well in soil extract, degradation of 1,3-D was not observed. Therefore, it appeared that the second carbon substrate that had the capacity to stimulate 1,3-D degradation was a heat-labile organic compound.

Table 10. Screening for second carbon substrates that stimulated degradation of cis- and trans-1,3-D by the mixed culture grown in basal mineral-trace mineral medium (BMTMM)[a].

Second carbon substrate	Degradation
Soil leachate (40 μL/mL)	+
Soil extract	-
glucose (200 μg/mL)	-
L-broth (Full strength)	-
L-broth (10 μL/mL)	+
Yeast extract (200 μg/mL)	-
Tryptone (200 μg/mL)	+
Tryptophan (200 μg/mL)	+

[a] With the exception of soil extract and full strength L-broth, the mixed culture was directly inoculated into the two media without using BMTMM.

It was costly and very difficult to obtain sufficient volume of soil leachate and, in addition, the mixed bacterial culture grew poorly and degraded 1,3-D slowly in BMTMM supplemented with soil leachate. By accident, it was observed that the mixed bacterial culture in BMTMM supplemented with a small amount of L-broth (10 μL/mL) rapidly degraded cis- and trans-1,3-D (Table 10), and also grew well in this medium. Although growth of the mixed culture in the full-strength L-broth was excellent, degradation of 1,3-D was not observed. L-broth consists of two organic components, yeast extract and tryptone. Yeast extract and tryptone (in BMTMM) thus were individually tested for their capacity to stimulate 1,3-D degradation by

the mixed culture (Table 10). Only tryptone had the capacity to stimulate 1,3-D degradation. Tryptophan is the dominant amino acid of tryptone (Difco Laboratories, 1953). Tryptophan in BMTMM was then found to have excellent capacity to stimulate 1,3-D degradation by the mixed culture, even better than tryptone. The mixed culture failed to degrade 1,3-D when grown in BMTMM supplemented with a readily degradable simple sugar, glucose, however.

1,3-Dichloropropene is subject to rapid chemical hydrolysis in aqueous media (McCall, 1987). The hydrolysis rate depends on temperature but is independent of pH at each temperature. Therefore, controls were always set up and incubated along with test culture media at the same time on the same shaker at 28°C. For those second carbon substrates capable of stimulating 1,3-D degradation, 1,3-D was completely degraded in 2 to 4 days by the mixed culture whereas, after 4 days, ≈60% of the 1,3-D remained in the controls. Trans-1,3-D was degraded faster than cis-1,3-D by the mixed culture if a suitable second carbon substrate was present, whereas the two isomers in the controls were degraded at the same rate. The mixed culture consisted of at least four species of bacteria, as distinguished from the morphology of their respective colonies.

DISCUSSION

Degradation of Cis- and Trans-1,3-D in Treated and Untreated Soils

This study has shown that a progressive increase in enhancement of cis- and trans-1,3-D degradation occurred for Arredondo soil from the treated Green Acres site following three consecutive annual field applications of 1,3-D in 1995, 1996, and 1997. As indicated before, this site had been treated with 1,3-D (Telone II) 6 times between 1982 and 1994, and in 1994, this site was treated with 1,3-D at a rate of 1.95 kg/ha. It is not known when enhanced degradation of 1,3-D began in soil at this site. However, it is known that degradation of 1,3-D in the soil was enhanced after the 1994 annual application (Ou et al., 1995). This study confirms the findings of Ou et al. (1995) that degradation of cis- and trans-1,3-D in soil from the treated site was enhanced, and that trans-1,3-D was degraded more rapidly than cis-1,3-D. It is not clear why lag phases for the degradation of cis- and trans-1,3-D were observed in surface and subsurface soils collected from the treated site 3 weeks after the 1995 annual application. No lag phases for degradation were observed with surface and shallow subsurface soils collected 3 weeks after the 1994 annual application from the same site (Ou et al., 1995). This site

received little rainfall for an extended period of time prior to 1995 soil collection. Lag phases were not observed for samples collected from the same site 3 weeks after the 1996 and 1997 annual applications. At present, 1,3-D may be the only known volatile fumigant and nematicide for which repeated applications result in enhanced degradation.

Obrigawitch et al.(1983) found that the degradation rates of the herbicide butylate increased progressively with each successive application during a period of three annual field applications of the chemical to Kennebec silt loam in Nebraska. Racke and Coats (1987) also found that, as the number of field applications of the insecticide isofenfos increased, degradation rates of the chemical were progressively increased. Recently, Trabue et al.(1997) demonstrated that the disappearance rates of carbofuran in enhanced soil progresssively increased during three consecutive annual applications of carbofuran to a Florida sandy soil. The degree of enhancement of 1,3-D degradation was greater for trans-1,3-D than for cis-1,3-D (See Figs. 9, 13, 16, and 17). Such differentially enhanced degradation was more markedly observed for the two upper layers of the treated soil than for the lowest layer of the soil collected three weeks after annual field applications of 1,3-D. It is likely that greater degradation of 1,3-D in the two upper layers of soil was due to stimulation of the activity of a 1,3-D-degrading microbial population of the treated site.

Telone II had been injected to the 15-20 cm depth below the surface. Since 1,3-D is volatile, microorganisms in the upper two layers of treated soil had a better chance to have interaction with 1,3-D than in the bottom layer of the soil.

The duration of enhanced degradation for a pesticide depends on soil type, soil properties such as physical, chemical, and biological characteristics, and climate (Bean et al., 1988). Therefore, long-term field studies are needed to evaluate the duration of enhancement of degradation for a pesticide under various soil types, and different climate regions. Due to difficulties in establishment of experimental plots for long-term field studies, little information is available on the period required in order for nematicide degradation rates in previously treated soils to return to those for untreated soils. Two studies have shown that the duration of enhanced degradation for fenamiphos is influenced greatly by climate. Ou (1991) reported that enhanced degradation of fenamiphos occurred after a single field application of the chemical to a site in north central Florida, a temperate region, with the enhancement lasting more than 3 years but less than 4 years. The duration of enhanced degradation for fenamiphos in tropical regions lasts about 18 months, however (J.P.E. Anderson, personal communication).

It is well-known that microorganisms incubated at an elevated temperature that is not high enough to inhibit them

have higher metabolic activities than those incubated at a lower temperature (Atlas and Bartha, 1986). Such increased respiratory activity reflects the marked effect of temperature on enzymes up to the temperature where protein denaturation occurs (Hargrave, 1969). McCall (1987) reported that degradation of 1,3-D in aqueous media increased with an increase in temperature, independent of pH. Therefore, it is expected that 1,3-D degrading microbial activity should be higher in early summer (June or July) than in winter (December or January) in Florida.

The control soils used for this long-term field study were collected in late spring or early summer, so microbial activity should be higher than for control soils collected in winters. For accurate determination and comparison of degradation rates of cis- and trans-1,3-D in treated and control soils, control soils and treated soils should be collected at the same time. The degradation rates of the two isomers in the control soils used for comparison with degradation rates in treated soils 6 and 10 months after the 1995 annual application, and 5 and 10 months after the 1997 annual application, thus may be higher than for actual control soils (if they had been collected at the same time as the treated soils).

Enhanced degradation of cis- and trans-1,3-D in treated surface and subsurface soils markedly decreased a few months after annual application. Two years after an annual

application, degradation rates of cis- and trans-1,3-D in the treated soils were nearly identical to those for the corresponding control soils. Differential degradation of cis- and trans-1,3-D had not been observed for the treated soils two years after an annual application. My study (1995 to 1997) showed that enhanced degradation of cis- and trans-1,3-D in the treated soil could persist for 2-3 years after the last field application of 1,3-D, even though the degradation rate by this time was nearly identical to that for the control soil. Eventually the degradation rates of the two isomers should return to rates similar to those for in soil that had no previous exposure to 1,3-D, however. Correspondingly, the degree of differential enhancement of cis- and trans-1,3-D degradation was diminished, even though the two isomers were still more rapidly degraded in the enhanced (treated) soil than in the nonenhanced (untreated) soil. Smelt et al. (1996) reported that the enhanced degradation of 1,3-D lasted more than 5 years after the last application to a study site (in 1987). No adequate control sites were established by Smelt et al. (1996), however. So-called untreated soils had been in fact treated 1 to 2 times 5 years earlier. Furthermore, the two soils used for the Dutch study were former marine deposits. Some marine microorganisms in these two soils might have retained the capacity to degrade 1,3-D regardless of the 1,3-D treatment history of the soils.

Most of the studies on enhanced degradation of 1,3-D have been conducted using soils from the Netherlands. A majority of these soils are of marine-sediment origin. Some microorganisms from these soils may retain their capacity to degrade halogenated compounds including 1,3-D. This was supported by the findings of Smelt et al. (1989, 1996), who observed that several untreated soils had the capacity to rapidly degrade 1,3-D regardless of the number of prior field applications of 1,3-D. Verhagen et al. (1995, 1996) used only one isomer, cis-1,3-D. Therefore, it is not known whether trans-1,3-D could be degraded faster than cis-1,3-D in formerly treated soil from the Netherlands.

One single field reapplication of 1,3-D to soil where 1,3-D had not been applied for two years resulted in the resumption of differential enhanced degradation of cis- and trans-1,3-D. This suggested that 1,3-D-degrading microorganisms were present in the soil even as much as two years after the last annual application. Three weeks after reapplication of 1,3-D, these microorganisms were apparently activated by 1,3-D, and produced the enzymes necessary for 1,3-D degradation.

In deeper subsurface (30-45 cm of depth) soil collected 3 weeks after an annual application of 1,3-D, differential enhanced degradation was not as great as for more-shallow soils. It was likely that little 1,3-D had moved into the lowest layer of soil to activate 1,3-D-degrading

microorganisms and produce the enzymes responsible for 1,3-D degradation.

Another question concerns why differential enhanced degradation of cis- and trans-1,3-D declined a few months after each annual application. Many enzymes are produced by soil microorganisms regardless of whether the optional substrates for these enzymes are present. These enzymes are known as constitutive enzymes. Other enzymes are produced only when appropriate substrates are present and available to the microorganisms. These are called inducible enzymes (Alexander, 1994; Neidhardt et al., 1990). When the 1,3-D-degrading microorganisms present in treated soil were stimulated by an annual field application of 1,3-D, they were induced to produce the enzyme necessary for 1,3-D degradation. It is likely that, 0.7 months after the each annual application, the induced enzyme activity should be the highest (see Figs. 9, 13, 16, and 17). Since degradation rates of cis- and trans-1,3-D in enhanced soil 5 to 6 months after the annual application had steadily declined, it was likely that the decline in degradation rates was due to a corresponding decline in the levels of enzymes responsible for 1,3-D degradation associated with 1,3-D-degrading bacteria, until eventually these enzymes were no longer present in the cells of the degraders. 1,3-D was completely degraded in enhanced soil by 30 days after 1,3-D

application, even in enhanced soil collected up to 10 months after an annual application (see Figs. 11 and 15). Therefore, in order for 1,3-D degraders in enhanced soil collected several months after annual application to degrade 1,3-D, enzymes responsible for 1,3-D degradation had to remain inducible. This assumption is supported by the fact that lag-phase periods (during which chemical degradation should predominate) for cis- and trans-1,3-D degradation in enhanced soil increased as collection-time intervals increased. For example, the lag-phase period for cis-1,3-D degradation in enhanced surface soil collected 3 weeks after the 1995 annual application was 3 days, whereas the lag-phase period for the same isomer in enhanced surface soil collected 10 months after the 1995 annual application was 7 days (see Figs. 9A and 11A).

The concentration of 1,3-D applied to soil was 40 μg/g, equivalent to 1.56 to 1.95 kg of Telone II/ha. 1,3-D in soil would be distributed in the soil solution phase, and also in the gaseous phase including soil pores and the headspace of centrifuge tubes. Considering that water solubilities of cis- and trans-1,3-D are 2180 and 2320 μg/mL with vapour pressures of 34.3 and 23.0 mm Hg, respectively (DowElanco, 1996), the majority of the two isomers should be in the solution phase. Ou (1989) reported that 1,3-D at 50 μg/g may inhibit microorganisms, resulting in a longer lag-phase for degradation in soil than when lower rates of 1,3-D are used.

Therefore, the previous study by Ou et al. (1995) and this study have demonstrated that 40 μg/g of 1,3-D in soil may suffice to induce differential enhanced degradation of 1,3-D by 1,3-D degrading microorganisms.

Simon et al. (1992) reported that higher microbial mass and higher organic matter content were positively correlated with pesticide degradation rates in soil. They found that microbial biomass and organic matter content were the major factors determining degradation of fenamiphos and fenamiphos sulfoxide. Similar results were reported by Chung and Ou (1996), who found that degradation rates of fenamiphos sulfoxide (FSO) and fenamiphos sulfone (FSO_2) in the surface layer of a soil were larger than in two underlying layers of soil. Therefore, higher organic matter contents in the two upper layers of soil used for this study may have contributed to higher microbial activity, resulting in more rapid degradation of 1,3-D than in the lowermost layer of soil.

1,3-D is a volatile fumigant, so recoveries of 80 to 95% of cis- and trans-1,3-D were reasonable. Without freezing of samples prior to extraction, recovery of ^{14}C-1,3-D was poor, ranging from 50 to 70% (Ou, 1989). Employment of a freezing technique in which solvent and soil samples were briefly stored in an ultra- cold freezer (-32°C) improved the mass recovery of cis- and trans-1,3-D from soil to 80 to 95%, which is acceptable.

Ou et al. (1995) detected a hydrolysis product of cis-1,3-D, cis-3-CAA (≤ 1.7 μg/g soil), and trace amounts of a hydrolysis product of trans-1,3-D, trans-3-CAA, in treated and untreated soils collected from the Green Acres site 3 weeks after the 1994 annual application. In the present study, a different GC technique was used for analysis of cis- and trans-3-CAA. Cis- and trans-3-CAA were occasionally detected in both treated and untreated soil samples. However, levels were generally below detection limits, and consistently below quantitation limits (data not shown). Judging from the findings of Ou et al. (1995) it was likely that, if a more sensitive GC method had been employed, the two hydrolysis products and especially cis-3-CAA might have been detected.

Comparing the half-life values of cis- and trans-1,3-D in enhanced soils with those in control and autoclaved soils, the major factor involved in enhanced degradation of the two isomers appears to be biological degradation, possibly initially through biological hydrolysis in enhanced soils. Chemical hydrolysis was also a major factor in autoclaved and nonenhanced soils. The initial step in biodegradation of 1,3-D is biological hydrolysis to 3-CAA (Lebbink et al., 1989; Roberts and Stoydin, 1976; Verhagen et al., 1995) rather than oxidation, which is responsible for the degradation of some short-chain halogenated hydrocarbons including TCE (Arciero et al., 1989; DiSpirito

et al., 1992) and methyl bromide (Oremland et al., 1994; Ou et al., 1997).

I did not find that the degradation of cis- and trans-1,3-D was accelerated in methane- and ammonium sulfate-treated soils (data not shown), even though methyl bromide in methanotrophic soils and ammonium sulfate-treated soils was rapidly degraded (Oremland et al., 1994; Ou et al., 1997). Therefore, methane monooxygenases and ammonia-monooxygenases appear to play no role in the degradation of cis- and trans-1,3-D. Rather, the two isomers appear to be initially degraded through hydrolysis.

Isolation of and Screening for Bacteria Capable of Degrading Cis- and Trans-1,3-D

Despite extensive efforts that have been made by Ou and his coworkers, microorganisms capable of utilizing cis- and trans-1,3-D as a sole source of carbon for growth could not be isolated from formerly treated soil. However, I did isolate a mixed bacterial culture capable of degrading cis- and trans-1,3-D from a treated soil. This culture degraded cis- and trans-1,3-D only in the presence of a second carbon substrate, such as tryptone or tryptophan. Therefore, the degradation appears to be a cometabolic process. Since tryptophan is the dominant amino acid of tryptone, it is understandable that either of these compounds can serve interchangeably as a second carbon substrate for the mixed bacterial culture which degrades cis- and trans-1,3-D. It is not clear why tryptophan is able to stimulate the mixed

culture to produce the necessary enzymes for degradation of the two isomers, because 1,3-D and tryptophan are not structurally related. Tryptophan consists of an indole and a side chain (alanine), whereas 1,3-D is a short-chain chlorinated hydrocarbon, consisting of a three-carbon chain. However, tryptophan can be microbially degraded to indole and alanine (Mallette et al., 1979). The role of indole and/or alanine thus needs to be tested for their capacity to stimulate the degradation of 1,3-D. Alanine may be the chemical of choice, since both 1,3-D and alanine consist of a three-carbon chain.

In the presence of soil leachate, the mixed bacterial culture had the capacity to degrade cis- and trans-1,3-D but not in the presence of soil extract. Thus, it is likely that the chemical(s) in the soil leachate that served as a second carbon substrate for the degradation of 1,3-D is/are heat-labile. Tryptophan is heat-labile, and is subject to acid hydrolysis, especially at high temperatures (Difco Laboratories, 1953). Since soil leachate may consist of a large number of organic chemicals, other chemical(s) may also serve as a second carbon substrate for the mixed culture to degrade 1,3-D as well.

Trans-1,3-D was degraded faster than cis-1,3-D both in enhanced soil and by the mixed culture. Thus, it is possible that the bacteria of the mixed culture might also be responsible for degradation of the two isomers in the soil.

At present, it is not known whether two different enzymes are responsible for the hydrolysis, with one being specific for cis-1,3-D and the other for trans-1,3-D, and/or if the one specific for trans-1,3-D has higher enzymatic activity than its counterpart. A single enzyme could also be responsible for the hydrolysis of both cis- and trans-1,3-D, but could have a higher activity toward trans-1,3-D, resulting in more rapid hydrolysis of the isomer.

I also entertained the idea that trans-1,3-D was isomerized to cis-1,3-D by microorganisms, resulting in more rapid degradation of the trans-1,3-D. However, I failed to validate this hypothesis. The (S)-fluazifop in soil was found to be enantiomerized to (R)-fluazifop (Bewick, 1986). Several soil bacteria have been found to have the capacity to utilize racemic 1,3-D or cis-1,3-D as a sole source of carbon for growth (Lebbink et al., 1989; Verhagen et al., 1995). However, these organisms were generally maintained in rich media. Thus, it is questionable that these bacteria could actually utilize 1,3-D or cis-1,3-D as a sole source of carbon for growth. These bacteria may instead cometabolize 1,3-D using some organic component of the medium as a second carbon substrate.

At present, little information is available about the genetics, molecular biology, and microbial ecology involved in the differential degradation of cis- and trans-1,3-D. Therefore, additional research toward understanding the

biodegradation of cis- and trans-1,3-D, and their key degradation products cis- and trans-3-CAA and cis- and trans-3-chloroacrylic acid, is needed.

CONCLUSIONS

First of all, repeated annual applications of 1,3-D to soil resulted in enhanced degradation of cis- and trans-1,3-D, with trans-1,3-D being degraded faster than cis-1,3-D. Second, this enhancement was progressively increased with an increase in the number of annual applications of 1,3-D. Third, enhanced degradation of cis- and trans-1,3-D was greater in treated surface (0 to 15 cm depth) and shallow subsurface (15 to 30 cm depth) soils than in treated deeper subsurface soil (30 to 45 cm depth). 1,3-D was injected into the soil 15 to 20 cm below the soil surface. Fourthly, enhanced degradation of cis- and trans-1,3-D in treated surface and subsurface soils markedly decreased a few months after each annual application. Two years after an annual application, degradation rates of cis- and trans-1,3-D in the treated soils were similar to those for the corresponding control soils. Differential degradation of cis- and trans-1,3-D was no longer observed in the treated soils two years after an annual application. Fifthly, microorganisms capable of utilizing 1,3-D as a sole source of carbon for growth could not be isolated from either enhanced or nonenhanced soils. Sixthly, unlike TCE and methyl bromide, degradation of cis- and trans-1,3-D was

not stimulated in methanotrophic soil and ammonium sulfate-treated soils. Seventhly, a mixed bacterial culture that had been isolated from an enhanced soil had the capacity to degrade 1,3-D. This mixed culture degraded 1,3-D in the presence of a second substrate such as tryptone and tryptophan, but not in the presence of glucose or of yeast extract. The mixed culture degraded trans-1,3-D faster than cis-1,3-D. Eighthly, it appeared that bacteria cometabolically and differentially degraded cis- and trans-1,3-D in enhanced soil.

REFERENCES

Alexander, M. 1977. Introduction to Soil Microbiology. John Wiley and Sons, Inc., New York, NY.

Alexander, M. 1981. Biodegradation of chemicals of environmental concern. Science 211:132-138.

Alexander, M. 1994. Biodegradation and Bioremediation. Academic Press, San Diego, CA.

Arciero, D., T. Vannelli, M. Logan, and A.B. Hooper. 1989. Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. Biochem. Biophys. Res. Commun. 159:640-643.

Atlas, R.M., and R. Bartha. 1972. Biodegradation of petroleum in seawater at low temperature. Can. J. Microbiol. 18:1851-1855.

Atlas, R.M., and R. Bartha. 1986. Microbial Ecology. 1987. The Benjamin/Cummings Publishing Company, Inc., New York, NY.

Audus, L.T. 1951. The biological detoxication of hormone herbicides in soil. Plant and Soil 3:170-191.

Avidov, E., N. Aharonson, and T. Katan. 1988. Accelerated degradation of diphenamid in soils and means for its control. Weed Sci. 36:519-523.

Bailey, A.M., and M.D. Coffey. 1985. Biodegradation of metalaxyl in Avocado soils. Phytopathology 75:135-137.

Baker, K.H., and D.S. Herson. 1994. Bioremediation. McGraw-Hill, Inc., New York, NY.

Barik, S., and D.M. Munnecke. 1982. Enzymatic hydrolysis of concentrated diazinon in soil. Bull. Environ. Contam. Toxicol. 29:235-239.

Barik, S., P.A. Wahid, C. Ramakrishna, and N. Sethunathan. 1979. A change in the degradation pathway of parathion after repeated applications to flooded soil. J. Agric. Food Chem. 27:1391-1392.

Barles, R.W., C.G. Daughton, and D.P.H. Hsieh. 1979. Accelerated parathion degradation in soil innoculated with acclimated bacteria under field conditions. Arch. Environ. Contam. Toxicol. 8:647-660.

Bayly, R.C., and M.G. Barbour. 1984. The degradation of aromatic compounds by the meta and gentisate pathways: Biochemistry and regulation. pp. 253-294. In Microbial Degradation of Organic Compounds (ed. D.T. Gibson). Marcel Dekker, Inc., New York and Basel.

Bean, B.W., F.W. Roeth, A.R. Martin, and R.G. Wilson. 1988. Duration of enhanced soil degradation of EPTC as influenced by herbicide rotation, time, and location. Weed Sci. 36:524-530.

Belser, N.O., and C.E. Castro. 1971. Biodehalogenation--The metabolism of the nematocides cis- and trans-3-chloroallyl alcohol by a bacterium isolated from soil. J. Agric. Food Chem. 19:23-26.

Bewick, D.W. 1986. Stereochemistry of fluazifop-butyl transformations in soil. Pestic. Sci. 17:349-356.

Butler, J.H. 1994. The potential role of the ocean in regulating atmospheric CH_3Br. Geophys. Res. Lett. 21:185-188.

Cain, R.B., and J.A. Mitchell. 1996. Enhanced degradation of the fungicide vinclozolin: Isolation and characterization of a responsible organism. Pestic. Sci. 48:13-23.

Caldwell, S.R., and F.M. Raushel. 1991. Detoxification of organophosphate pesticides using a nylon based immobilized phosphotriesterase from *Pseudomonas diminuta*. Appl. Biochem. Biotechnol. 31:59-73.

Camacho, R.L., L.J. Moshier, and J.E. Rodebush. 1981. Comparison of thiocarbamate herbicides applied alone and in combination with fonofos or R-33865 for shattercane control in corn with EPTC history. Proc. North Cent. Weed Control Conf. 36:128-129.

Camper, N.D., M.M. Fleming, and H.D. Skipper. 1987. Biodegradation of carbofuran in pretreated and non-pretreated soils. Bull. Environ. Contam. Toxicol. 39:571-578.

Castro, C.E., and N.O. Belser. 1966. Hydrolysis of cis- and trans-1,3-dichloropropene in wet soil. J. Agric. Food Chem. 14:69-70.

Chapalamadugu, S., and G.R. Chaudhry. 1991. Hydrolysis of carbaryl by a *Pseudomonas* sp. and construction of a microbial consortium that completely metabolizes carbaryl. Appl. Environ. Microbiol. 57:744-749.

Chaudhry, G.R., and S. Chapalamadugu. 1991. Biodegradation of halogenated organic compounds. Microbiol. Rev. 55:59-79.

Chung, K.Y., and L.T. Ou. 1996. Degradation of fenamiphos sulfoxide and fenamiphos sulfone in soil with a history of continuous applications of fenamiphos. Arch. Environ. Contam. Toxicol. 30:452-458.

Clarke, P.H. 1984. The evolution of the degradation pathways. pp. 11-27. In Microbial Degradation of Organic Compounds (ed. D.T. Gibson). Marcel Dekker, New York, NY.

Dalton, H. 1992. Methane oxidation by methanotrophs: Physiological and mechanistic implications. pp. 85-113. In Methane and Methanol Utilizers (eds. J.C. Murrell and H. Dalton).

Dalton, H., S.D. Prior, D.J. Leak, and S.H. Stanley. 1984. Regulation and control of methane monooxygenase. pp.75-82. In Microbial Growth on C1 Compounds (eds. R.L. Crawford and R.S. Hanson). Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, D.C.

Daughton, C.G., and D.P.H. Hsieh. 1977. Accelerated parathion degradation in soil by inoculation with parathion-utilizing bacteria. Bull. Environ. Contam. Toxicol. 18:48-56.

Dick, W.A., R.O. Ankumah, G. Mcclung, and N. Abou-Assaf. 1990. Enhanced degradation of EPTC (s-ethyl N,N-dipropylcarbamothioate) in soil and by an isolated soil microorganism. pp 98-112. In Enhanced Biodegradation of Pesticides in the Environment (eds. K.D. Racke and J.R. Coats). ACS Symp. Ser. 426. American Chemical Society, Washington, D.C.

Difco Laboratories. 1953. Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. 9th ed. Difco Laboratories Inc., Detroit, MI.

DiSpirito, A.A., J. Gulledge, A.K. Shiemke, J.C. Murrell, M.E. Lidstrom, and C.L. Krema. 1992. Trichloroethylene oxidation by the membrane-associated methane monooxygenase in type I, type II and type X methanotrophs. Biodegradation 2:151-164.

DowElanco. 1996. 1,3-Dichloropropene: A profile. DowElanco, Indianapolis, IN.

Dowler, C.C., L.S. Marti, and C.S. Kvien. 1984. Degradation rates of butylate and alachlor following repeated field treatments. Proc. South. Weed Sci. Soc. 37:323.

Drozd, J.W. 1980. Respiration in the ammonia oxidizing chemotrophic bactia. Vol. 2. pp. 87-111. In Diversity of Bacterial Systems (ed. C.J. Knowles). CRC Press, Inc., Boca Raton, FL.

Duffy, J.R., and N. Wong. 1967. Residues of organochlorine insecticides and their metabolites in soils in the Atlantic provinces of Canada. J. Agric. Food Chem. 15:457-464.

Eagle, D.J. 1986. ADAS experience of enhanced degradation of carbofuran. Aspects of Appl. Biol. 13:101-105.

Elliott, S., and F.S. Rowland. 1993. Nucleophilic substitution rates and solubilities for methyl halides in seawater. Res. Lett. 20:1043-1046.

Ellis, C.R. 1982. A survey of granular application equipment and insecticide rates used for control of corn rootworms (Coleoptera: Chrysomelidae) in southern Ontario. Proc. Entomol. Soc. Ont. 113:29-34.

Elsner, E., D. Bieniek, W. Klein, and F. Korte. 1972. Chemosphere 1:247-250.

Ensley, B.D. 1991. Biochemical diversity of trichloroethylene metabolism. Ann. Rev. Microbiol. 45:283-299.

Felsot, A.S. 1989. Enhanced biodegradation of insecticides in soil. Implications for agroecosystems. Annu. Rev. Entomol. 34:453-479.

Felsot, A.S, J.V. Maddox, and W. Bruce. 1981. Enhanced microbial degradation of carbofuran in soils with histories of furadan use. Bull. Environ. Contam. Toxicol. 26:781-788.

Felsot, A.S., J.G. Wilson, D.E. Kuhlman, and K.L. Steffey. 1982. Rapid dissipation of carbofuran as a limiting factor in corn rootworm (Coleoptera:Chrysomelidae) control in fields with histories of continuous carbofuran use. J. Econ. Entomol. 75:1098-1103.

Ferguson, W., and A. Padula. 1994. Economic effects of banning methyl bromide for soil fumigation. USDA Economic Research Service, Agricultural Economic Report No. 677. USDA, Beltville, MD.

Fetzner, S., and F. Lingens. 1994. Bacterial dehalogenase: Biochemistry, genetics, and biotechnological applications. Microbiol. Rev. 58:641-685.

Fogel, M.M., A.R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.

Fryer, J.D., P.D. Smith, and R.J. Hance. 1980. Field experiments to investigate long-term effects of repeated applications of MCPA, triallate, simazine, and linuron: II. Crop performance and residues 1969-1978. Weed Res. 103-110.

Fukimori, F., and R.P. Hausinger. 1993a. *Alcaligenes eutrophus* JMP134 2,4-dichlorophenoxyacetate monooxygenase is an α-ketoglutarate-dependent dioxygenase. J. Bacteriol. 175:2083-2086.

Fukimori, F., and R.P. Hausinger. 1993b. Purification and characterization of 2,4-dichlorophenoxyacetate/α-ketoglutarate dioxygenase. J. Biol. Chem. 268:24311-24317.

Gan, J., S.R. Yates, M.A. Anderson, W.F. Spencer, F.F. Ernst, and M.V. Yates. 1994. Effect of soil properties on degradation and sorption of methyl bromide in soil. Chemosphere 29:2685-2700.

Geiger, W., and E. Molner-Kubica. 1977. Tetrachloroethylene in ground and drinking waters. Bull. Environ. Contam. Toxicol. 19:475-580.

Gentile, I.A., L. Ferraris, and S. Crespi. 1989. The degradation of methyl bromide in some natural fresh waters. Influence of temperature, pH, and light. Pestic. Sci. 25:261-272.

Getzin, L.W., and I. Rosefield. 1968. Organophorous insecticide degradation by heat-labile substances in soil. J. Agric. Food Chem. 16:598-601.

Getzin, L.W., and C. Shanks. 1990. Enhanced degradation of carbofuran in Pacific Northwest soils. J. Environ. Sci. Health B25:433-446.

Gibson, W.P., and R.G. Burns. 1977. The breakdown of malathion in soil and soil components. Microbial Ecol. 3:219-230.

Guenzi, W.D., and W.E. Beard. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science 156:1116-1117.

Guenzi, W.D., and W.E. Beard. 1968. Anaerobic conversion of DDT to DDD and aerobic stability of DDT in soil. Soil Sci. Soc. Am. J. 32:522-524.

Guenzi, W.D., and W.E. Beard. 1976. DDT degradation in flooded soil as related to temperature. J. Environ. Qual. 5:391-394.

Gunsolus, J.L., and R.S. Fawcett. 1980. Decreasing effectiveness of EPTC in continuous use fields: Confirmation and possible chemical and cultural remedies. Proc. North Cent. Weed Control Conf. 35:18.

Gunsolus, J.L., and R.S. Fawcett. 1981. Accelerated degradation of EPTC + R-25788 in fields of continuous EPTC + R-25788 use. Proc. North Cent. Weed Control Conf. 36:24.

Harder, W. 1981. Enrichment and characterization of degrading organisms. pp. 77-96. In Microbial Degradation of Xenobiotics and Recalcitrant Compounds. (eds. T. Leisinger, R. Hutter, A.M. Cook and J. Neusch). Academic Press, New York, NY.

Hargrave, B.T. 1969. Epibenthic algal production and community respiration in the sediments of a marine lake. J. Fisheries Res. Board of Canada 26:2003-2026.

Harris, C.R., R.A. Chapman, C. Harris, and C.M. Tu. 1984. Biodegradation of pesticides in soil: Rapid induction of carbamate degrading factors after carbofuran treatment. J. Environ. Sci. Health B19:1-11.

Harris, C.R., R.A. Chapman, R.F. Morris, and A.B. Stevenson. 1988. Enhanced soil microbial degradation of carbofuran and fensulfothion - A factor contributing to the decline in effectiveness of some soil insect control programs in Canada. J. Environ. Sci. Health B23:301-316.

Harvey, R.G., and D.B. Schuman. 1981. Accelerated degradation of thiocarbamate herbicides with repeated use. Abstr. 21:124. Weed Science Society of America.

Hassall, K. 1982. The Chemistry of Pesticides. The Macmillan Press, Ltd., London, UK.

Higgins, I.J., D.J. Best, and R.C. Hammond. 1980. New findings in methane-utilizig bacteria highlight their importance in the biosphere and their commercial potential. Nature 286:561-564.

Horng, L.C., and D.D. Kaufman. 1987. Accelerated biodegraation of several organophosphate insecticides. Abstr. 193rd National Meeting of American Chemical Society, Denver, CO.

Horvath. 1972. Cometabolism. Bacteriol. Rev. 36:146-155.

Hubaux, A. and G. Vos. 1970. Decision and detection limits for linear calibration curves. Anal. Chem. 42:849-855.

Hyman, M.R., and P.M. Wood. 1983. Methane oxidation by *Nitrosomonas europaea*. Biochem. J. 212:31-37.

Hyman, M.R., and P.M. Wood. 1984. Ethylene oxidation by *Nitrosomonas europaea*. Arch. Microbiol. 137:155-158.

Hyman, M.R., A.W. Sansome-Smith, J.H. Shears, and P.M. Wood. 1985. A kinetic study of benzene oxidation to phenol by whole cells of *Nitrosomonas europaea* and evidence for further oxidation of phenol to hydroxyquinone. Arch. Microbiol. 143:302-306.

Infante, P.F., and T.A. Tsongas. 1982. Mutagenic and oncogenic effects of chloromethanes, chloroethanes, and halogenated analogs of vinyl chloride. Environ. Sci. Res. 25:301-327.

Iwata, Y., M. Ittig, and F.A. Gunther. 1977. Degradation of O,O- dimethyl S-[α-(carboethoxy benzyl]phosphoro dithioate (phenthoate) in soil. Arch. Environ. Contam. Toxicol. 6:1-21.

Jones, R.D., and R.Y. Morita. 1983. Carbon monoxide oxidation by chemolithotrophic ammonium oxidizers. Can. J. Microbiol. 29:1545-1551.

Jury, W.A., and M. Ghodrati. 1989. Overview of organic chemical environmental fate and transport modeling approaches. pp. 271-304. In Reactions and Movement of Organic Chemicals in Soil (eds. B.L. Sawhney and K. Brown). Soil Science Society of America, Madison, WI.

Kaufman, D.D., and D.F. Edwards. 1983. Pesticide/microbe interaction effects on persistence of pesticides in soil. Vol. 4. pp. 177-182. In Pesticide Chemistry: Human Welfare and the Environment. (eds. J. Miyamoto and P.C. Kearney). Pergamon, Inc., Oxford, UK.

Kaufman, D.D., Y. Katan, D.F. Edwards, and E.G. Jordan. 1985. Microbial adaptation and metabolism of pesticides. pp 437-451. In Agricultural Chemicals of the Future (ed. J.L. Hilton). BARC (Beltsville Agric. Res. Cent.) Symp. No. 8. Rowman/Allanheld, Totowa, NJ.

Khalil, M.A.K., R.A. Rasmussen, and R. Gunawardena. 1993. Atmospheric methyl bromide: Trends and global masss balance. J. Geophys. Res. 98:2887-2896.

Kim, C.J., and W.J. Mayer. 1986. Acclimation and biodegradation of chlorinated organic compounds in the presence of alternate substrates. J. Water Pollut. Control Fed. 58:157-164.

Lebbink, G., B. Proper, and A. Nipshagen. 1989. Accelerated degradation of 1,3-dichloropropene. Acta Horticulturae 255:361-371.

Leistra, M., A.E. Groen, S.J.H. Crum, and L.J.T. Van der Pas. 1991. Transformation rate of 1, 3- dichloropropene and 3-chloroallyl alcohol in topsoil and subsoil material of flower-bulb fields. Pestic. Sci. 31:197-207.

Lichtenstein, E.P., and K.R. Schultz. 1960. Epoxidation of aldrin and heptachlor in soils as influenced by autoclaving, moisture, and soil types. J. Econ. Entomol. 53:192-197.

Lichtenstein, E.P., and K.R. Schultz. 1964. The effects of moisture and microorganisms on the persistence and metabolism of some organophosphorous insecticides in soils, with special emphasis on parathion. J. Econ. Entomol. 57:618-627.

Little, C.D., A.V. Palumbo, S.E. Herbes, M.E. Lidstrom, R.L. Tyndall, and P.J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing acterium. Appl. Environ. Microbiol. 54:951-956.

Love, O.T. Jr., and R.G. Eilers. 1982. Treatment of drinking water containing trichloroethylene and related industrial solvents. J. Am. Water Works Assoc. 74:413-425.

Maddy, K.T., H.R. Fong, J.A. Lowe, D.W. Conrad, and A.S. Fredrickson. 1982. A study of well water in selected California communities for residues of 1,3-dichloropropene, chloroallyl alcohol and 49 organophosphate or chlorinated hydrocarbon pesticides. Bull. Environ. Contam. Toxicol. 29:354-359.

Mallette, M.F., C.O. Clagett, A.T. Phillips, and R.L. McCarl. 1979. Introductory Biochemistry. Robert E. Krieger Publishing Co., Hungtington, NY.

Martin, C., D. Vega, J. Bastide, and P. Davet. 1990. Enhanced degradation of iprodione in soil after repeated treatments for controlling Sclerotinia minor. Plant and Soil 127:140-142.

McCall, P.J. 1987. Hydrolysis of 1,3-dichloropropene in dilute aqueous solution. Pestic. Sci. 19:235-242.

McDougall, J., and J. Woodburn. 1989. Agrochemical Review. County Natwest Woodmac, London, UK.

Meyers, A.J. 1980. Evaluation of bromoethane as a suitable analogue in methane oxidation studies. FEMS Microbiol. Lett. 9:297-300.

Munnecke, D.M. 1979. Hydrolysis of organophosphate insecticides by an immobilized enzyme system. Biotechnol. Bioeng. 22:2247-2261.

Munnecke, D.M., and D.P.H. Hsieh. 1976. Pathways of microbial metabolism of parathion. Appl. Environ. Microbiol. 31:63-69.

Nannipieri, P., and J.M. Bollag. 1991. Use of enzymes to detoxify pesticide-contaminated soils and waters. J. Environ. Qual. 20:510-517.

Neidhardt, F.C., J.L. Ingraham, and M. Schaechter. 1990. Physiology of the Bacterial Cell. Sinauer Associates, Inc., Sunderland, MA.

Niemczyk, H.D., and R.A. Chapman. 1987. Evidence of enhanced degradation of isofenfos in turfgrass thatch and soil. J. Econ. Entomol. 80:880-882.

Noling, J.W., and J.O. Becker. 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. Suppl. J. Nematol. 26: 573- 586.

Obrigawitch, T., A.R. Martin, and F.W. Roeth. 1980. Breakdown of EPTC and EPTC plus R-33865 on a conditioned and nonconditioned soil. Proc. North Cent. Weed Control Conf. 35:20.

Obrigawitch, T., R.G. Wilson, A.R. Martin, and F.W. Roeth. 1982. The influence of temperature, moisture and prior EPTC application on the degradation of EPTC in soils. Weed Sci. 30:175-181.

Obrigawitch, T., A.R. Martin, and F.W. Roeth. 1983. Degradation of thiocarbamate herbicides in soils exhibiting rapid EPTC breakdown. Weed Sci. 31:187-192.

Oremland, R.S., L.G. Miller, C.W. Culbertson, T.L. Connell, and L. Jahnke. 1994. Degradation of methyl bromide by methanotrophic bacteria in cell suspension and soils. Appl. Environ. Microbiol. 60:3640-3646.

Ott, L. 1977. An introduction to statistical mthods and data analysis. Wadsworth Publishing Co., Belmont, CA.

Ou, L.T. 1989. Degradation of Telone II in contaminated and noncontaminated soils. J. Environ. Sci. Health B24 6:661-674.

Ou, L.T. 1991. Interactions of microorganisms and soil during fenamiphos degradation. Soil Sci. Soc. Am. J. 55:716-722.

Ou, L.T., and J.E. Thomas. 1994. Influence of organic matter and soil surfaces on a bacterial consortium that mineralizes fenamiphos. Soil Sci. Soc. Am. J. 58:1148-1153.

Ou, L.T., D.H. Gancarz, W.B. Wheeler, P.S.C. Rao, and J.M. Davidson. 1982. Influence of soil temperature and soil moisture on degradation and metabolism of carbofuran in soil. J. Environ. Qual. 11:293-298.

Ou, L.T., K.S.V. Edvardsson, and P.S.C. Rao. 1985a. Aerobic and anaerobic degradation of aldicarb in soils. J. Agric. Food Chem. 33:72-78.

Ou, L.T., K.S.V. Edvardsson, J.E. Thomas, and P.S.C. Rao. 1985b. Aerobic and anaerobic degradation of aldicarb sulfone in soils. J. Agric. Food Chem. 33:545-548.

Ou, L.T., J.E. Thomas, and D.W. Dickson. 1994. Degradation of fenamiphos in soil with a history of continuous fenamiphos applications. Soil Sci. Soc. Am. J. 58:1139-1147.

Ou, L.T., K.Y. Chung, J.E. Thomas, T.A. Obreza, and D.W. Dickson. 1995. Degradation of 1,3-dichloropropene (1,3-D) in soils with different histories of field applications of 1,3-D. J. Nematol. 27:249-257.

Ou, L.T., P.J. Joy, J.E. Thomas, and A.G Hornsby. 1997. Stimulation of microbial degradation of methyl bromide in soil during oxidation of an ammonia fertilizer by nitrifiers. Environ. Sci. Tech. 31:717-722.

Palleroni, N.J. 1995. Microbial versatility. pp. 3-25. In Microbial Transformation and Degradation of Toxic Organic Chemicals (eds. L.Y. Young and C.E. Cerniglia). Wiley-Liss, Inc., New York, NY.

Petura, J.C. 1981. Trichloroethylene and methylchloroform in groundwater: A problem assessment. J. Am. Water Works Assoc. 73:200-205.

Prather, M.J., and R.T. Watson. 1990. Stratospheric ozone depletion and future levels of atmospheric chlorine and bromine. Nature (London) 344:729-734.

Racke, K.D., and J.R. Coats. 1987. Enhanced degradation of isofenfos by soil microorganisms. J. Agric. Food Chem. 35:94-99.

Racke, K.D., and J.R. Coats. 1988. Enhanced degradation and the comparative fate of carbamate insecticides in soil. J. Agric. Food Chem. 36:1067-1072.

Racke, K.D., and J.R. Coats. 1990. Enhanced Biodegradation of Pesticides in the Environment. ACS Symp. Ser. 426. American Chemical Society, Washington, D.C.

Rahman, A., and T.K. James. 1983. Decreased activity of EPTC + R-25788 following repeated use in some New Zealand soils. Weed Sci. 31:783-789.

Rahman, A., G.C. Atkinson, J.A. Douglas, and D.P. Sinclair. 1979. Eradicane causes problems. New Zealand J. Agric. 139:47-49.

Rajagopal, B.S., S. Panda, and N. Sethunathan. 1986. Accelerated degradation of carbaryl and carbofuran in a flooded soil pretreated with hydrolysis products, 1-naphthol and carbofuran phenol. Bull. Environ. Contam. Toxicol. 36:827-832.

Rasche, M.E., M.R. Hyman, and D.J. Arp. 1990. Biodegradation of halogenated hydrocarbon fumigants by nitrifying bacteria. Appl. Environ. Microbiol. 56:2568-2571.

Read, D.C. 1983. Enhanced microbial degradation of carbofuran and fensulfothion after repeated applications to acid mineral soil. Agric. Ecosyst. Environ. 10:37-46.

Read, D.C. 1986. Influence of weather conditions and microorganisms on persistence of insecticides to control root maggots (Diptera: Anthomyiidae) in rutabagas. Agric. Ecosyst. Environ. 16:165-173.

Read, D.C. 1987. Greatly accelerated microbial degradation of aldicarb in retreated field soil, in flooded soil, and in water. J. Econ. Entomol. 80:156-163.

Reineke, W. 1984. Microbial degradation of halogenated aromatic compounds. pp. 319-360. In Microbial Degradation of Organic Compounds (ed. D.T. Gibson). Marcel Dekker, Inc., New York, NY.

Roberts, T.R., and G. Stoydin. 1976. The degradation of (Z)- and (E)-1,3-dichloropropenes and 1,2-dichloropropane in soil. Pestic. Sci. 7:325-335.

Roeth, F.W. 1984. Effects of enhanced biodegradation on weed control. Agrochem. Div. Abstr. No. 94. American Chemical Society, Washington, D.C.

Roeth, R.W. 1986. Enhanced herbicide degradation in soil with repeated application. Rev. Weed Sci. 2:46-76.

Rudyanski, W.J., R.S. Fawcett, and R.S. McAllister. 1987. Effect of prior pesticide use on thiocarbamate herbicide persistence and giant foxtail (Setaria faberi) control. Weed Sci. 35:68-74.

SAS Institute, Inc. 1989. SAS User's Guide:Statistics, SAS System Version 6. 4th edition. SAS Institute, Inc., Cary, NC.

Schmidt, E., M. Hellwig, and H.-J. Knackmuss. 1983. Degradation of chlorophenols by a defined mixed microbial community. Appl. Environ. Microbiol. 46:1038-1044.

Schuman, D.B., and R.G. Harvey. 1980. Predisposition of soils for rapid thiocarbamate herbicide breakdown. Proc. North Cent. Weed Control Conf. 35:19-20.

Sethunathan, N., and T. Yoshida. 1973. Parathion degradation in submerged rice soils in the Philippines. J. Agric. Food Chem. 21:504-506.

Sethunathan, N., R. Siddaramappa, K.P. Rajaram, S. Barik, and P.A. Wahid. 1977. Parathion: Residues in soil and water. Res. Rev. 68:91-122.

Simon, L., M. Spiteller, A. Haisch, and P.R. Wallofer. 1992. Influence of soil properties on the degradation of the nematocide fenamiphos. Soil Biol. Biochem. 24:769-773.

Skujins, J. 1976. Vol. 4. pp. 383. In Extracellular Enzymes in Soil (eds. A.I. Laskin and H. Lechevalier). CRC Critical Rev. in Microbiol. CRC Press, Cleveland, OH.

Smelt, J.H., S.J.H. Crum, W. Teunissen, and M. Leistra. 1987. Accelerated transformation of aldicarb, oxamyl and ethoprophos after repeated soil treatments. Crop Protection 6:295-303.

Smelt, J.H., W. Teunissen, S.J.H. Crum, and M. Leistra. 1989. Accelerated transformation of 1,3-dichloropropene in loamy soils. Netherlands J. Agric. Sci. 37:173-183.

Smelt, J.H., A.E. Van De Peppel-Groen, L.J.T. Van Der Pas, and A. Dijksterhuis. 1996. Development and duration of accelerated degradation of nematicides in different soils. Soil Biol. Biochem. 28:1757-1765.

Smith, A.E., and A. Walker. 1977. A quantitative study of asulam persistence in soil. Pestic. Sci. 8:449-456.

Spain, J.C., P.H. Prictchard, and A.W. Bourqin. 1980. Effect of adaptation on biodegradation rates in sediment/water cores from estuarine and fresh water environments. Appl. Environ. Microbiol. 40:726-734.

Steffey, K., D. Kuhlman, and K. Kinney. 1987. Corn rotworm larval control-research and management in Illinois. pp. 50-62. In Proc. IL. Agric. Pestic. Conf., Urbana-Champaign, IL.

Stephenson, T., J.N. Lester, and R. Perry. 1984. Acclimatization to nitrilotriacetic acid in the activated sludge process. Chemosphere 13:1033-1040.

Suett, D.L. 1986. Accelerated degradation of carbofuran in previously treated field soils in the United Kingdom. Crop Prot. 5:165-169.

Suett, D.L. 1987. Influence of treatment of soil with carbofuran on the subsequent performance of insecticides against cabbage root fly (Delia radicum) and carrot fly (Psila rosae). Crop Prot. 6:371-378.

Suett, D.L., and A.A. Jukes. 1988. Accelerated degradation of aldicarb and its oxidation products in previously treated soils. Crop Protection. 7:147-152.

Suwa, Y., A.D. Wright, F. Fukumori, K.A. Nummy, R.P. Hausinger, W.E. Holben, and L.J. Forney. 1996. Characterization of a chromosomally encoded 2,4-Dichlorophenoxyacetic acid/α-ketoglutarate dioxygenase from Burkholderia sp. Strain RASC. Appl. Environ. Microbiol. 62:2464-2469.

Thomson, W.T. 1986. Agricultural Chemicals. Vol. I. Herbicides. Fresno, CA.

Tollefson, J.J. 1986. Why soil insecticidal immunity? Solutions 30:48-55.

Top, E.M., O.V. Maltseva, and L.J. Forney. 1996. Capture of catabolic plasmid that encodes only 2,4-Dichlorophenoxyacetic acid: α-Ketoglutaric acid dioxygenase (TfdA) by genetic complementation. Appl. Environ. Microbiol. 62:2470-2476.

Trabue, S., X. Feng, A. Ogram, and L.T. Ou. 1997. Carbofuran degradation in soil profile. J. Environ. Sci. Health. B32:861-878.

Turco, R.F., and A. Konopka. 1990. Biodegradation of carbofuran in enhanced and non-enhanced soil. Soil Biol. Biochem. 22:195-201.

Turpin, F.T., and A.C. York. 1981. Insect management and the pesticide syndrome. Environ. Entomol. 10:567-572.

U.S. Environmental Protection Agency. 1985. Substances found at proposed and final NPL sites through update number three. Document NPL-U3-6-3. U.S. Environmental Protection Agency, Washington, D.C.

Van der Meer, J. R., W.M. De Vos, S. Harayama, and A.J.B. Zehnder. 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. 1992. Microbiol. Rev. 56:677-694.

Van der Pas, L.J.T., and M. Leistra. 1987. Movement and degradation of 1,3-dichloropropene in the soil of flower-bulb fields. Arch. Environ. Contam. Toxicol. 16:417-422.

Van Dijk, H. 1974. Degradation of 1,3-dichloropropenes in the soil. Agro-Ecosystems 1:177-184.

Van Dijk, H. 1980. Dissipation rates in soil of 1,2-dichloropropane and 1,3- and 2,3-dichloropropenes. Pestic. Sci. 11:625-632.

Van Waarde, J.J., R. Kok, and D.B. Janssen. 1993. Degradation of 2-chloroallyl alcohol by a *Pseudomonas* sp. Appl. Environ. Microbiol. 59:528-535.

Varma, M.M., L.W. Wan, and C. Prasad. 1976. Acclimation of wastewater bacteria by induction or mutation selection. J. Water Pollut. Control Fed. 48:832-834.

Ventullo, R.M., and R.J. Larson. 1986. Adaptation of aquatic microbial communities to quaternary ammonium compounds. Appl. Environ. Microbiol. 51:356-361.

Verhagen, C., E. Smit, D.B. Janssen, and J.D. Van Elsas. 1995. Bacterial dichloropropene degradation in soil: Screening of soils and involvement of plasmids carrying the dhlA gene. Soil Biol. Bichem. 27:1547-1557.

Verhagen, C., G. Lebbink, and J. Bloem. 1996. Enhanced biodegradation of the nematicides 1,3-dichloropropene and methyl isothiocyanate in a variety of soils. Soil Biol. Biochem. 28:1753-1756.

Voet, D. and J. Voet. 1990. Biochemistry. John Wiley and Sons, Inc., New York, NY.

Vogel, T.M., and P.L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49:1080-1083.

Vosey, P.A., and P.M. Wood. 1987. Methanol and formaldehyde by an autotrophic nitrifying bacterium. J. Gen. Microbiol. 33:283-290.

Walker, A. 1974. A simulation model for prediction of herbicide persistence. J. Environ. Qual. 3:396-401.

Walker, A. 1976a. Simulation of herbicide persistence in soil. I. Simazine and prometryne. Pestic. Sci. 7:41-49.

Walker, A. 1976b. Simulation of herbicide persistence in soil. II. Simazine and linuron in long-term experiments. Pestic. Sci. 7:50-58.

Walker, A. 1978. Simulation of the persistence of eight soil applied herbicides. Weed Res. 18:305-313.

Walker, A., and A.E. Smith. 1979. Persistence of 2,4,5-T in a heavy clay soil. Pestic. Sci. 10:151-157.

Walker, A., P.A. Brown, and A.R. Entwistle. 1986. Enhanced degradation of iprodione and vinclozolin in soil. Pestic. Sci. 17:183-193.

Watson, R.T., D.L. Albritton, S.O. Anderson, and S. Lee-Bapty. 1992. Methyl bromide: Its atmospheric science, technology, and economics. United Nations Environmental Program, United Nation Headquarters, Nairobi, Kenya.

Westrick, J.J., J.W. Mello, and R.F. Thomas. 1984. The groundwater supply survey. J. Am. Water Works Assoc. 76:52-59.

Wiggins, B.A., S.H. Jones, and M. Alexander. 1987. Explanations for the acclimation period preceding the mineralization of organic chemicals in aquatic environments. Appl. Environ. Microbiol. 53:791-796.

Wilde, G., and T. Mize. 1984. Enhanced microbial degradation of systemic pesticides in soil and its effects on chinch bug Blissus leucopterus (say) (heteroptera: Lygaeidae) and greenbug Schizaphis graminum Rondani (Homoptera: Aphididae) control in seedling sorghum. Environ. Entomol. 13:1079-1082.

Williams, I.H., H.S. Pepin, and M.J. Brown. 1976. Degradation of carbofuran by soil microorganisms. Bull. Environ. Contam. Toxicol. 15:244-249.

Wilson, R.G. 1984. Accelerated degradation of thiocarbamate herbicides in soil with prior thiocarbamate herbicide exposure. Weed Sci. 32:264-268.

Wilson, J.T., and B.H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

Yang, R.S.H. 1986. 1,3-Dichloropropene. Res. Rev. 97:19-35.

Yon, D.A., G.A. Morrison, and A.S. McGibbon. 1991. The dissipation of 1,3-dichloropropene in ditch bottom sediment and associated aerobic ditch water. Pestic. Sci. 32:147-159.

Zublena, J.P., D.T. Gooden, and H.D. Skipper. 1987. Influence of crop-chemical rotations on enhanced biodegradation. Agron. Abstr. pp. 195. American Society of Agronomy, Madison, WI.

APPENDIX
NUMERICAL DATA OBTAINED FROM GC ANALYSIS

Table A-1. Degradation of cis- and trans-1,3-D in treated soil 3 weeks after the 1995 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	16.4	15.4	14.6	15.8	16.4	15.2
3	14.9	12.7	12.3	10.7	11.2	14.2
5	14.0	12.4	11.2	1.8	2.5	10.5
7	0.1	0.2	10.8	0.0	0.0	8.9
10	0.0	0.0	7.9	0.0	0.0	2.6
14	0.0	0.0	0.0	0.0	0.0	0.0

Table A-2. Degradation of cis- and trans-1,3-D in control soil 3 weeks after the 1995 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	15.6	16.8	14.1	16.6	17.9	15.3
3	12.4	10.5	10.7	12.2	10.5	10.7
7	7.6	8.1	10.0	7.1	7.3	9.9
14	2.7	3.0	6.7	0.8	1.4	6.1
21	0.0	0.0	-	0.0	0.0	-
28	0.0	0.0	4.4	0.0	0.0	1.2

Table A-3. Degradation of cis- and trans-1,3-D in treated soil 6 months after the 1995 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	20.1	19.6	19.1	18.8	18.4	17.5
3	12.1	12.0	12.8	12.5	12.1	13.1
7	10.1	10.7	12.1	8.7	8.3	11.5
10	7.0	5.2	9.1	2.6	0.1	8.7
14	0.7	0.5	7.8	0.1	0.0	6.2
21	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.o	0.0

Table A-4. Degradation of cis- and trans-1,3-D in treated soil 10 months after the 1995 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	16.9	17.9	17.6	17.8	17.6	16.4
3	12.4	11.2	12.3	13.0	11.2	11.9
7	5.8	6.3	8.2	4.9	5.3	7.3
14	0.5	2.8	6.2	0.2	0.4	4.3
21	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0

Table A-5. Degradation of cis- and trans-1,3-D in treated soil 2 years after the 1995 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	18.9	18.5	17.9	18.8	18.4	17.8
3	11.1	11.1	11.5	11.0	10.7	11.2
7	8.2	7.1	10.0	8.1	6.9	9.6
14	5.6	5.3	7.3	5.6	4.8	6.3
21	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0

Table A-6. Degradation of cis- and trans-1,3-D in treated soil 3 weeks after the 1996 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	17.5	18.0	16.8	17.9	19.0	16.0
3	12.1	12.4	14.4	7.3	7.3	13.4
5	3.9	3.1	-	0.3	0.2	-
7	0.2	0.0	9.4	0.0	0.0	6.9
10	0.2	0.0	-	0.0	0.0	-
14	0.0	0.0	0.5	0.0	0.0	0.1
21	0.0	0.0	0.0	0.0	0.0	0.0

Table A-7. Degradation of cis- and trans-1,3-D in control soil 3 weeks after the 1996 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	16.3	17.1	15.6	17.3	18.4	16.5
3	11.3	11.8	11.0	11.1	11.6	10.8
7	5.5	4.9	7.7	4.5	3.9	6.1
14	3.8	4.9	7.5	2.9	4.1	5.9
21	2.3	3.1	5.0	1.7	2.5	4.2
28	1.1	0.2	4.6	0.6	0.1	3.7

Table A-8. Degradation of cis- and trans-1,3-D in treated soil 5 months after the 1996 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)		Trans-1,3-dichloropropene (μg/g)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
0	16.4	17.1	16.8	17.4
3	11.0	11.4	9.9	10.0
7	6.3	7.3	4.3	5.5
14	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0

Table A-9. Degradation of cis- and trans-1,3-D in treated soil 10 months after the 1996 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	16.7	16.0	14.4	16.3	16.3	16.1
3	11.1	11.4	11.2	12.1	10.7	11.2
7	6.3	8.4	8.0	6.0	6.9	6.6
14	0.1	0.4	5.2	0.0	0.0	3.7
21	0.0	0.0	2.8	0.0	0.0	1.1
28	0.0	0.0	0.0	0.0	0.0	0.0

Table A-10. Degradation of cis- and trans-1,3-D in treated soil 3 weeks after the 1997 annual field application.

Time (days)	Cis-1,3-dichloropropene (μg/g)			Trans-1,3-dichloropropene (μg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	20.4	19.9	18.7	19.1	19.0	18.4
3	10.2	9.9	11.5	6.0	4.9	10.2
5	1.6	1.7	8.6	0.0	0.0	5.1
7	0.0	0.0	4.2	0.0	0.0	0.1
10	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0	0.8

Table A-11. Degradation of cis- and trans-1,3-D in control soil 3 weeks after the 1997 annual application.

Time (days)	Cis-1,3-dichloropropene (µg/g)			Trans-1,3-dichloropropene (µg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	18.3	16.6	17.0	18.0	16.5	17.0
3	12.7	13.1	9.9	12.7	11.8	9.4
7	9.4	8.0	7.7	9.0	7.7	6.9
14	6.2	5.6	4.5	5.8	4.5	3.2
21	2.9	4.3	3.7	2.7	3.5	2.5
28	0.0	3.5	2.5	0.0	2.5	1.7

Table A-12. Degradation of cis- and trans-1,3-D in treated soil 3 weeks after reapplication to soils that had not been treated with 1,3-D for 2 years.

Time (days)	Cis-1,3-dichloropropene (µg/g)			Trans-1,3-dichloropropene (µg/g)		
	0-15 cm	15-30 cm	30-45 cm	0-15 cm	15-30 cm	30-45 cm
0	20.0	17.6	18.8	19.9	17.4	19.0
3	10.1	12.9	11.5	7.3	10.3	10.4
5	8.0	8.4	-	1.7	3.5	-
7	1.5	4.1	10.3	0.0	0.1	7.9
10	0.0	0.0	4.6	0.0	0.0	1.3
14	0.0	0.0	0.0	0.0	0.0	0.0
21	-	-	0.0	-	-	0.0

Table A-13. Degradation of cis- and trans-1,3-D in autoclaved surface and subsurface soils 5 months after the 1996 annual application.

Time (days)	Cis-1,3-dichloropropene (μg/g)		Trans-1,3-dichloropropene (μg/g)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
0	18.3	18.3	18.4	18.1
3	11.1	11.3	10.7	10.3
7	7.8	7.9	7.3	7.1
14	5.9	7.1	5.3	6.5
21	5.2	6.0	5.0	5.3
28	3.5	4.0	3.1	3.7

BIOGRAPHICAL SKETCH

Keun-Yook Chung was born in Jae Cheon, Chung Buk, Korea. He attended Dongmyong Primary School, Jaecheon Middle School, and Kyunggi High School. After high school graduation, he briefly attended Chung Buk National University in Cheongju, Korea and then served as a soldier attached to the publication and education unit in the national army for 3 years. After returning to college, he was awarded a 4-year scholarship given to excellent students by the Chung Buk National University. During this time, he led the Time magazine club, which was a study group for learning and improving skills in contemporary English including writing, reading, and speaking. He was awarded a Bachelor of Science in Agriculture (BSA) degree from the Department of Agricultural Chemistry in February, 1987. He was eager to study soil microbiolgy with an emphasis on the biodegradation of pesticides in the environment because his country has serious environmental pollution problems from the disposal of pesticides and other toxic organic chemicals. The problem is compounded by the lack of expert scientists in this area of endeavour. He came to the U.S.A. to take courses in preparation for entering this research field. He was accepted for graduate study with his major

advisor, Dr. Li-Tse Ou, a soil microbiologist in the Department of Soil and Water Science at the University of Florida in August, 1991, and was awarded the degree of Master of Science in December, 1993. He then entered a Ph.D. program in the area of biodegradation and microbial ecology under the supervision of Dr. Ou. He will be formally awarded the degree of Doctor of Philosophy in May, 1998.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Li-Tse Ou, Chair
Scientist of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Brian L. McNeal
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Peter Nkedi-Kizza
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ann C. Wilkie
Research Assistant Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Willis B. Wheeler
Emeritus Professor of Food Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1998

Dean, College of Agriculture

Dean, Graduate School